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INVESTIGATION OF TWO INSECT SPECIES FOR CONTROL OF EURASIAN WAT--ETC(U)
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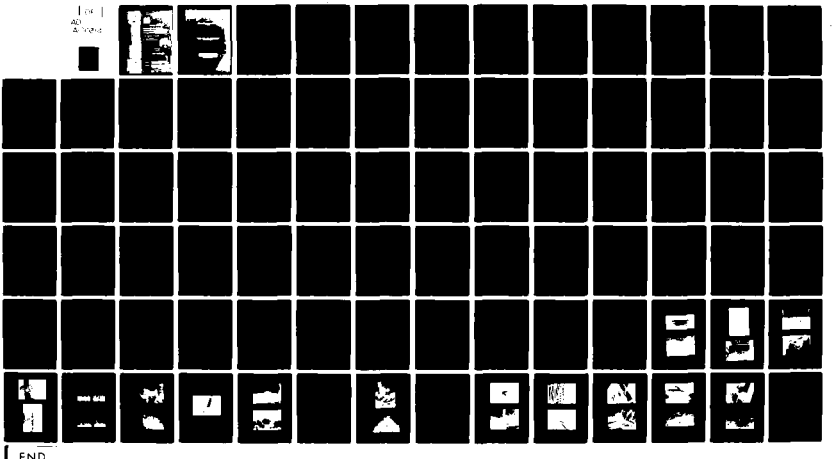

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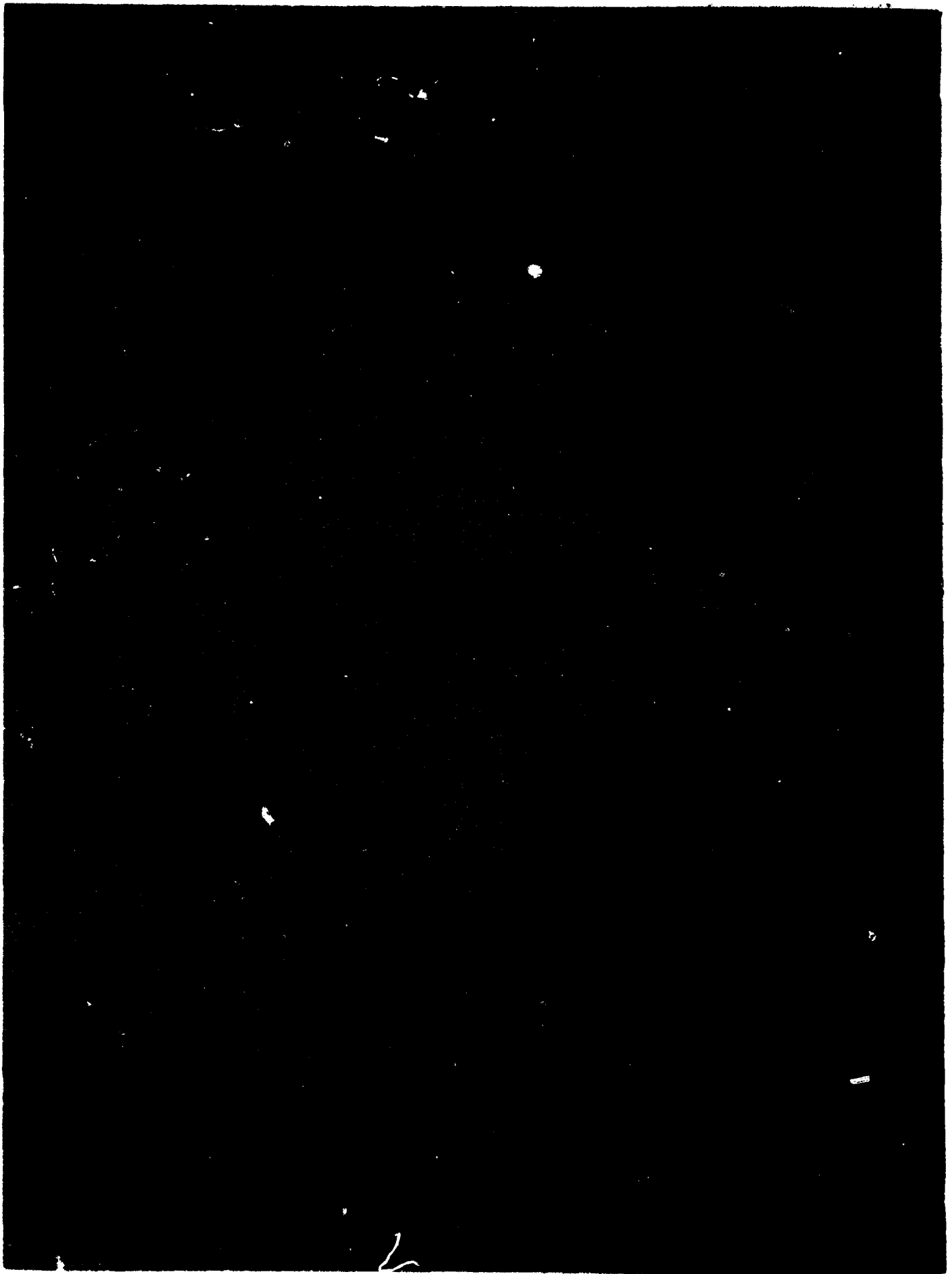

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This report presents the results of the evaluation of two species of insects for the biological control of Eurasian watermilfoil (Myriophyllum spicatum L.). One of the species is a native weevil, Litodactylus leucogaster (Marsh.), which attacks the flower spikes, and the other is an accidentally introduced European moth, Acentria nivea (Olivier), whose larvae feed on the leaves and stems. The biology of L. leucogaster was studied in detail and

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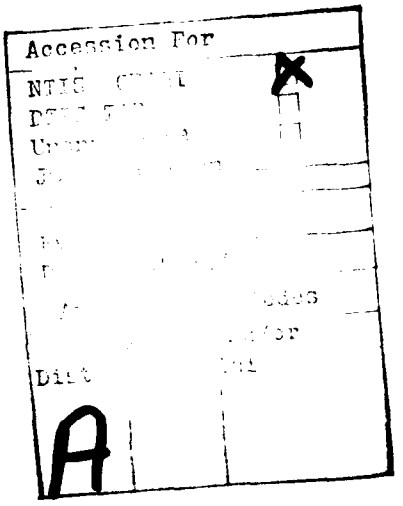
host-specificity tests were conducted with both adults and larvae. Since it was determined to be specific to watermilfoils, it was released at Crystal River, Florida, on 22 August 1979. Two adults were captured at Crystal River in November indicating possible establishment. The pyralid moth, A. nivea, was difficult to rear in the laboratory. Since the females were active only one night, successful matings were rare in the small colony. Larval production was best in containers that were minimally disturbed. The larvae required water temperatures below 22°C for development though they could survive short periods at higher temperatures. The larvae were not specific to milfoil; however, A. nivea may still be of interest as a member of a complex of species which attack milfoil.



PREFACE

This report presents results of a biological control program being conducted for the Aquatic Plant Control Research Program (APCRP) by the U. S. Department of Agriculture (USDA), Science and Education Administration (SEA), Biocontrol Laboratory, Gainesville, Fla. The purpose of this program was to evaluate insects to determine their potential for use in aquatic plant control. Funds for this effort were provided by the Office, Chief of Engineers, under appropriation number 96X3122, Construction General, through the APCRP at the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg,Miss.

The principal investigator for the work was Dr. Gary R. Buckingham, USDA, who prepared this report. He was assisted in the conduct of the work and preparation of the report by Mmes. Chris A. Bennett and Bonnie M. Ross. The authors are indebted to the following persons: Dr. Kenneth R. Langdon and Mr. Carlos Artaud, Florida Department of Agriculture; Dr. Pat Warrington, British Columbia Ministry of Environment, Water Investigations Branch, Victoria; Mr. Gilbert Bendix, San Francisco Water Department, Millbrae, Calif.; Dr. Ted Center and Dr. Suzanne Batra, Agricultural Research, SEA, USDA; Dr. Joseph Balciunas, University of Florida, Institute of Food and Agricultural Sciences, Department of Entomology and Nematology; and Ms. Marian Cousineau, Robert Moses State Park, Massena, N. Y. The authors also wish to thank the Florida Department of Agriculture, Gainesville, for providing the research facilities; Knox Boat House, Crystal River, Fla., for aiding the field collections; and Black and Cannon Realty, Crystal River, for providing tide tables.

The research was monitored at WES by Dr. D. R. Sanders, Sr., and Mr. R. F. Theriot of the Environmental Laboratory (EL), Wetland and Terrestrial Habitat Group (WTHG). The study was conducted under the general supervision of Dr. John Harrison, Chief, EL, Dr. C. J. Kirby, Jr., Chief, Environmental Resources Division, and the direct supervision of Dr. H. K. Smith, Acting Group Chief, WTHG. Mr. J. L. Decell is Manager of the APCRP at WES.

Commanders and Directors of WES during the study and the preparation of this report were *COL John L. Cannon, CE, and COL Nelson P. Conover, CE.* Technical Director was Mr. F. R. Brown.

This report should be cited as follows:

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INVESTIGATION OF TWO INSECT SPECIES FOR CONTROL OF EURASIAN WATERMILFOIL

PART I: INTRODUCTION

1. Eurasian watermilfoil (Myriophyllum spicatum L.), herein called milfoil, is a submersed perennial macrophyte that was introduced into the United States in the late 1800's, probably from Europe. Reed (1977) and Aiken et al. (1979) have discussed both the history of its spread and the distribution in the United States and Canada. Since it is a highly competitive species, it replaces native plants and forms large surface mats (Figure 1) which impede boats, interfere with recreation, and provide breeding areas for mosquitoes.

2. Milfoil is rooted in the hydrosoil and grows to the surface where the long stems float and grow along the surface. Aerial flower spikes, about 6-10 cm tall, are often produced in abundance by these surface stems (Figure 2). The spike has whorls of female flowers basally and whorls of male flowers apically (Figure 3). Perfect flowers are sometimes found between the two sections. Four seeds are produced by each female flower. After flowering the plant fragments and then usually regrows and flowers again. Seeds germinate in the laboratory but seedlings have not been found in nature (Aiken et al. 1979). This suggests that germination might be inhibited by an existing plant population and occurs only in new habitats. Stem sections root easily and account for the large increase in plants once a waterway is invaded.

3. In North America milfoil is the primary weedy species in the genus Myriophyllum, but the three natives, Northern watermilfoil (M. exalbescens Fern.), variable leaf milfoil (M. heterophyllum Michx.), and green milfoil (M. verticillatum L.), and the introduced parrot-feather (M. aquaticum (Velloso) Verdc.) are also occasional weeds. Milfoil, Northern watermilfoil, and green milfoil are closely related and are difficult to distinguish. There are approximately 20 species of Myriophyllum in North America (Muenscher, 1944) and 40 species worldwide (Cook, 1974). They are included in the family Haloragaceae (=Haloragidaceae) along with four other genera, only one of which is found in North America (Cook, 1974). This other genus, Proserpinaca, the mermaidweeds, is also aquatic. Another North American genus, Hippuris, mare's tail, has often been included in the Haloragaceae but Cook (1974) separates it into its own family. The families most closely related to the Haloragaceae are the Lythraceae (loosestrifes) and the Onagraceae (water primroses).

4. Surveys for insects which might have potential for biological control of milfoil have been conducted in Pakistan, Bangladesh, and Yugoslavia for the U.S. under PL480 contracts. Habib-ur-Rehman et al. (1969) listed 11 insect species associated with Myriophyllum spp. in Bangladesh and Pakistan. Baloch et al. (1972) reported studies with the four insect species which appeared most promising. Two of these were Pakistani weevils in the genus Bagous, which is related to the waterhyacinth weevils, Neochetina. These Bagous appeared to be host specific but the larval stages developed only in emersed milfoil

growing on wet banks. This behavior would apparently restrict their development in the U.S. to drawdown situations. A third weevil, Phytobius sp., and a gelechid moth, Aristotelia sp., developed on the flowers of M. indicum and M. tuberculatum in Bangladesh. These two insect species were specific in the field, although Phytobius sp. developed on water pepper (Polygonum hydropiper L.) in the laboratory.

5. Lekic and Mihajlovic (1970) found 15 insect species associated with milfoil in Yugoslavia. Most of these species were not specific or were rare and their biologies were not studied. Larvae of Bagous longitarsus Thoms. were found on submersed plants, unlike those species of Bagous in Pakistan, but the populations of B. longitarsus were too small to study. Two other weevil species, Eubrichiopsis velatus (Beck) and Litodactylus leucogaster (Marsham), were specific to milfoil and both are already present in the U.S. One of the Yugoslavian moths, Acentria nivea (Olivier) (=Acentropus niveus), is also present in the U.S. Another moth, Parapoynx stratiotata L., was recommended for further studies by Lekic and Mihajlovic, but Dr. Dale Habeck, University of Florida, Gainesville, determined that it was not specific after testing it in Rome, Italy.

6. Since L. leucogaster is native to North America (see Appendix A), it was chosen by use for further study. Investigations on its laboratory biology, behavior, and host specificity are reported here.

7. The North American specimens were described as Phytobius griseomicans Schwarz but they were synonymized by Dieckmann (1972) with L. leucogaster based upon a specimen from Alberta, Canada.

Dieckmann also stated that a second North American species, P. albertanus (Brown), might be a synonym of L. leucogaster. Only one additional species of Litodactylus has been described and that is L. testaceus Motsch. from Ceylon (Dalla Torre & Hustache, 1930). Its host plant has not been reported. L. leucogaster has a holarctic distribution, being found throughout Europe, in Central Siberia, and in North America (Dieckmann, 1972). Previously published North American locations are Alberta (Canada), Dakota, Kansas, Michigan, Washington, and Wisconsin (Dieckmann, 1972; Kissinger, 1964; Leng, 1920; Schwarz, 1892).

8. The genus Litodactylus is in the subfamily Ceutorhynchinae, tribe Ceutorhynchini, and other closely related U.S. genera are Eubrychiopsis (=Eubrychius), Mecopeltus, Pelenomus, Perenthis, Perigaster, Phytobius, and Rhinoncus (Dieckmann, 1972; Kissinger, 1964; Leng, 1920).

9. The host plants of L. leucogaster in Europe are M. spicatum and M. verticillatum (Dieckmann, 1972) and in North America it has been found on M. spicatum and probably on the native M. exalbescens Fernald which is the most prevalent species of milfoil in the areas where the weevil occurs. There has been confusion regarding the uniqueness of the latter plant species, so that merely milfoil or M. spicatum is usually listed as the host. M. verticillatum also occurs in North America in much of its range and is undoubtedly a host. Host plant genera reported for the seven closely related weevil genera are Myriophyllum, Potamogeton, Ludwigia, Polygonum, and Rumex with the exception of one European species of Phytobius, P. comari Herbst.,

which has been reported on Comarum and Lythrum. These plant genera may not all be true hosts, and the validity of Potamogeton as a host for some species is questionable since no one has actually reported larvae on that plant. Although the host plants for the majority of species are still unreported, this group of weevil genera appears to have a narrow range of hosts.

10. The pyralid moth, A. nivea, which was mentioned previously, is native to Europe and was apparently accidentally introduced into North America. It was first collected at Montreal, Quebec, Canada, in 1927 (Sheppard, 1945) and was subsequently found most often along the St. Lawrence River or in the general vicinity of Lakes Ontario and Erie (Judd, 1950). Dr. S. W. T. Batra, Agricultural Research, SEA, USDA, Beltsville, Maryland, collected larvae in 1977 in Ontario, Canada, at White Lake which is in the Ottawa River drainage system (personal communication). Mr. C. P. Kimball of Barnstable, Massachusetts, collected adults at Barnstable in 1949 (personal communication) and both he and Treat (1954, 1955) have other records for that state. Batra (1977) reported museum specimens collected at Middleton, Wisconsin, in 1963 and Kimball has specimens collected at Bailey's Harbor, Door County, Wisconsin, in 1966. See Appendix B.

11. The other members of the small pyralid subfamily to which A. nivea belongs, the Schoenobiinae, are associated with emersed and semiaquatic plant species in the Poaceae (Graminae) and the Cyperaceae, for example, Phragmites, Glyceria, Scirpus, Carex, and Eleocharis. The larvae of these other species are internal borers and are not truly aquatic like those of A. nivea.

12. The biology of A. nivea has been thoroughly studied in Europe (Berg, 1942; Nigmann, 1908; Ritsema, 1878) and Batra (1977) studied it in the U.S. As a result of her studies, Batra concluded that the species might have potential for use in Florida against both milfoil and hydrilla (Hydrilla verticillata (L. fil) Royle) but that further host-specificity studies were necessary. Field observations and host-specificity studies are reported here.

PART II: METHODS AND MATERIALS

Studies with L. leucogaster

Shipping and rearing

13. Overwintering adult weevils were shipped from California via air freight by Mr. Robert W. Pemberton, Berkeley, California. They were enclosed in unwaxed cardboard screwtop mailing tubes containing wood excelsior that had been soaked in water and then allowed to dry until damp. No plant material was included. The parents of all weevils used in the studies came from Lake Pilarcitos, San Mateo County, California.

14. Milfoil buds and flower spikes with about 15-20 cm of attached stem were collected weekly to bimonthly from June-October, 1978-1979, at Crystal River, Florida. Most collections were made at or near Bagley Cove. They were held in the laboratory in water-filled plastic pans in temperature cabinets at about 10-12°C until used in the rearing or experiments. The cool temperatures prevented them from maturing too rapidly.

15. The laboratory colony was maintained in a large wooden cage in a greenhouse. This cage consisted of a basal box, about 2.4 m square and 0.27 m deep sitting on legs about 0.9 m long. The box was waterproofed with multiple coats of liquid fiberglas and attached to it was a 1-m-high wooden frame, covered on the sides with nylon organdy and on the top with a translucent fiberglas panel. Sleeve openings in the center of each side allowed access to the cage. New flower spikes and buds with the attached stem were added periodically to the cage and

the old ones were removed. In 1978 water was occasionally allowed to overflow in the cage in order to clean it but better results were obtained in 1979 by maintaining a constant overflow. The greenhouse temperature was maintained at about 26°C; however, it occasionally surpassed 35°C during equipment failures.

16. Large numbers of weevils were also reared in 3.8-l (1-gal) glass jars covered with nylon organdy. Flower spikes and buds with the attached stem were also used in the jars. The jars were half filled with water and were usually initiated with about 10 flower spikes and 2-3 pairs of weevils. As the larvae developed, additional flowers were added and the water was changed occasionally until a large number of pupae were present. The stems were then removed from the water and held in jars containing damp paper towels until the adults emerged. Stems with pupae from the large cage were also handled often in this manner. Most jars were held in a windowless rearing room at about 24°C with fluorescent grolux lights on a 12-or 16-hr light cycle.

17. Rearing in smaller containers such as 50-dram plastic vials and quart (0.94-l) jars was attempted but was not very successful. Apparently excessive humidity is detrimental since larvae could be reared in open vials better than when they were covered with organdy or plastic tops. The humidity in organdy-covered quart jars was apparently okay but the jars did not hold sufficient plant material for efficient rearing.

Biology and behavior

18. Fecundity tests were conducted by confining newly emerged pairs in 50-dram plastic vials covered with nylon organdy. The vials were half

filled with water and several small pieces of styrofoam were added to provide resting spots for the beetles. The flowers were observed under a microscope before testing to insure that no field-deposited eggs of other weevils were present. One or two flower spikes with 3-5 cm of attached stem were added at each change. After 1-3 days exposure, the flowers were again checked for eggs under the microscope. Clean vials were used at each change and the old ones were soaked in bleach to prevent breakdown of the plants by microorganisms. If a male died it was replaced with a new one. At various intervals eggs were dissected from the plant and held on moist cotton in 30-ml (1-oz) plastic cups covered with cardboard lids in order to determine egg viability. The eggs plus 10 of the vials were kept in the rearing room with the glass jars. An additional five vials were kept in the greenhouse.

19. Mature larvae were placed on flower spikes with short stem sections in water-filled culture tubes in order to determine the length of the prepupal, pupal, and teneral adult stages. These stages could usually be observed without disturbance through the cocoon walls by transmitted light. It was necessary, however, to remove the water and make a small hole in some of the cocoons in order to observe the contents. The tubes were kept in a laboratory room at 24-27°C with natural lighting from a side window with supplemental fluorescent lighting from about 0700-1730. Fifty-dram plastic vials containing flower stalks with eggs and larvae were also kept in the laboratory room to determine the length of the larval stadia.

20. Adults were placed in ice-cooled water in order to slow down their movements for better observation of swimming techniques. Measurements were made at 12x, 25x, or 50x with a Wild M5 Stereomicroscope equipped with an ocular micrometer in 10x eyepieces. An Ehrenreich Photo Optical Industries, MK II, fiber optic light was used as the light source. Egg, larval, and pupal measurements were made with living specimens. Voucher specimens of adults and immatures have been deposited in the Florida State Collection of Arthropods, Gainesville, and in the U.S. National Museum, Washington, D.C.

Host specificity

21. Adult starvation tests were conducted with plant species related to milfoil, host plants of closely related weevils, or plant species important in or near the aquatic environment. Plants in the feeding tests were also checked for oviposition. The plant species tested for oogenesis and larval survival were chosen from among those that the feeding tests indicated might be at risk.

22. Adult starvation tests were conducted during 1978-1979 depending upon weevil and test plant availability. Either 1, 2, or 3 pairs of adults were used per container with 3-5 replications. Some plant species were also replicated in time. A series of miscellaneous flowers found near aquatic habitats was tested in 1978 with no replication and 2 pairs of adults per 50-dram plastic vial. Most starvation tests were conducted with field-collected beetles although some newly emerged beetles were also tested. These field-collected beetles were emerging from hibernation on the shore when captured and would be the stage most likely to encounter non-host plants. Since both aquatic and terrestrial plants and both

bouquets and potted plants were tested, the techniques varied. In order to describe them more easily, the techniques have been grouped into the following types:

Type 1 - 50-dram plastic vials filled with water and covered with nylon organdy. Flower spike or stem section of aquatic plant tested.

Type 2 - 0.95-1 (1-qt) glass jar with a shallow layer of water in the bottom and covered with nylon organdy. Stem section of terrestrial plant tested.

Type 3 - 0.47-1 (1-pt) unwaxed paper cup with a clear plastic lid. Bouquet of stems or flowers in a water-filled vial inserted through a hole in the side of the cup.

Type 4 - 14.5-cm-ID plexiglas cylinder covered with nylon organdy and placed over potted test plant.

Type 5 - 50-dram plastic vial placed over the stem tip of a potted test plant and plugged with cotton.

Type 6 - 14.5-cm-ID plexiglas cylinder covered with nylon organdy and placed in the large water-filled cage in the greenhouse. Flower or stem section of aquatic plant tested.

23. Tests of Types 1-5 were conducted in the rearing room. The plants in the starvation tests were checked for eggs as well as for feeding damage. Newly emerged females were checked for oogenesis in both Type 1 and Type 6 tests and newly emerged, or neonate, larvae were tested for survival in mostly Type 1 tests but also in Type 5 tests.

Studies with Acentria nivea (Olivier)

Shipping and rearing

24. Larvae were collected on northern watermilfoil in the St. Lawrence River at Lake St. Lawrence, Robert Moses State Park, near

Massena, New York, in June and September, 1978. The June collections were made in front of the Barnhart Marina and the adjacent public bathing beach. The September collections were made at various locations in the vicinity of the Long Sault Dam and the Barnhart Marina. The plant material was collected in June by snorkeling with full wet suits. In September it was collected from a boat by hand or by using rakes. The larval shelters were picked from the field-collected plants and placed into plastic containers containing small amounts of stems. The stems with the newly formed shelters were placed into heavy plastic bags on the nights before departure. In June they were carried for a day by auto. Small battery-operated minnow bucket aerators were used in the evening after the auto trip to aerate the water in the bags which were floated in cold water in a bathtub. They were aerated several times in the evening and once early in the morning before the morning flight to Gainesville. The aerators were also used the night before departure in September. The plastic bags were hand-carried on commercial flights during both trips.

25. Various methods were used to maintain a colony in quarantine for 1-½ years. The insects were held in aquaria of various sizes or in 3.8-l glass jars. Initially they were placed in a laboratory room with natural lighting plus supplemental fluorescent lighting. The temperature was about 24°C. Later they were removed to a rearing room where the temperature was maintained at about 25°C and there was only fluorescent lighting. The final and most successful procedure was to hold them in a temperature cabinet at 18-22°C with a 16L:8D photoperiod. The containers were occasionally aerated with air stones attached to aquarium

pumps and some of the water was exchanged. The larvae were fed the Crystal River milfoil.

26. Specimens of adults and larvae in alcohol have been deposited in the Florida State Collection of Arthropods, Gainesville.

Host specificity

27. No-choice tests with the larger larvae (10-13 mm) collected in June were conducted by placing individual larvae in culture tubes, 150 x 20 mm, containing about a 10-cm section of test plant stem. The tubes were covered by a piece of nylon organdy held in place by a plastic cap and were kept in the rearing room with the colony. The degree of feeding was evaluated subjectively because of the various shapes of the test plant leaves. Thus, feeding approximately equal to that on milfoil was designated moderate (equal to about 50% of the milfoil leaf material being eaten) and feeding including only a few feeding spots with no appreciable damage to the plant stem was designated minor. The duration of the test was two weeks and the stems of the species being tested were changed biweekly. At the end of the test the larvae were added to the rearing colony. The following plant species were tested (number of larvae used in parentheses): alligatorweed (Alternanthera philoxeroides (Martius) Grisebach) (3); fanwort (Cabomba caroliniana Gray) (3); slender spikerush (Eleocharis acicularis (L.) Roem. & Schult.) (4); hydrilla (5); water pennywort (Hydrocotyle umbellata L.) (3); frogbit (Limnobium boscii Rich) (2); creeping waterprimrose (Ludwigia repens Forst.) (3); parrotfeather (Myriophyllum aquaticum (Velloso) Verdc.) (2); Eurasian watermilfoil (5); southern naiad (Najas guadalupensis (Sprengel) Magnus) (6); watercress (Rorippa nasturtium-aquaticum (L.) Hayek) (2); Illinois

pondweed (Potamogeton illinoensis Morong) (2); mermaidweed (Proserpinaca palustris L.) (2); mermaidweed (Proserpinaca pectinata Lam.) (2); salvinia (Salvinia rotundifolia Willd.) (3); cattail (Typha sp.) (1); dwarf arrowhead (Sagittaria subulata (L.) Buchenau) (4).

28. Although actual choice tests were not conducted, stems of hydrilla, southern naiad, Illinois pondweed, and coontail (Ceratophyllum demersum L.) were mixed with those of milfoil in some of the colony rearing jars with the June larvae. In order to determine potential development on species other than milfoil, 20 small larvae (3-4 mm) from the September collection were placed into each of five jars containing individually either hydrilla, Illinois pondweed, coontail, Brazilian elodea (Egeria densa Planchon), or milfoil. Also one egg batch obtained from the colony in spring 1979 was placed in a jar with Illinois pondweed to determine if the newly emerged larvae (neonates) could develop on it. A second species of pondweed (P. perfoliatus L.) was added later, when the larvae were larger, because of a shortage of Illinois pondweed.

PART III: RESULTS AND DISCUSSION

Studies with Litodactylus leucogaster

Field collections and observations

29. Adult weevils were collected 17 April-6 June 1978 from milfoil debris along the shore of Lake Pilarcitos in California by Mr. Pemberton. The location and collecting technique were suggested by Dr. Charles W. O'Brien, Florida A & M University, Tallahassee. According to Mr. Pemberton, the early collections were made from debris above the high water line, but the latter ones were made from fresh debris near the water's edge. The weevils were picked from the milfoil debris by hand since various techniques such as using heat or immersing the debris did not force the beetles out. The following four shipments were received in Gainesville:

a. FBCL-78-5*, approximately 175 adults, 10 dead on arrival

b. FBCL-78-6, approximately 167 adults, 21 dead

c. FBCL-78-7, approximately 200-400 adults, 10 dead

d. FBCL-78-8, approximately 608 adults, 13 dead

A small number of weakened adults died within a few days of each shipment. A sample of the dead beetles plus some freshly killed beetles were examined for pathogens by Mr. Gerard Thomas, University of California, Insect Diagnostic Laboratory, Berkeley, but none were present. This shipping method was highly satisfactory, at least for the 1-2 days of transit time.

30. Mr. Pemberton and Dr. Lloyd Andres, Agricultural Research, SEA/USDA, Albany, California, collected beetles at Lake Pilarcitos on 21 May 1979. One

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* Shipment Receipt Number, Florida Biological Control Laboratory (Gainesville).

shipment was sent on 30 May using the same techniques as in 1978. This shipment was:

> RWP-79-1* (FBCL-79-3), 445 adults (187 females, 258 males), 54 dead.

31. On 20 October 1978, Lake Pilarcitos was visited to search for adults and larvae of L. leucogaster. The lake is a small manmade reservoir which is managed by the San Francisco Water Department and is south of San Francisco on the peninsula. There was about a 3- to 4-m fringe of milfoil around the shore of one arm of the lake with an extensive mat at the end of the arm. During three hours of searching at midday, no larvae and only two adults (one of each sex) were found. These adults were sitting on stems below flower spikes and may have been submersed before the stems were lifted for observation. There were many submersed mature flower spikes but only scattered emersed ones. Empty cocoons and feeding damage on the old flower spikes suggested that there had been a larger population but that the adults had already flown to shore for hibernation. Several large bags of submersed stems as well as some dry stems from shore were collected and held in a greenhouse for several days but no adults were recovered. A heavy infestation of aphids was observed on the flower spikes and along the emersed portions of the stems. Flowers of a pondweed (Potamogeton sp.) were inspected for signs of L. leucogaster feeding but none were seen. Two nearby lakes, San Andreas Lake and Crystal Springs Reservoir, were also infested by milfoil but they were not searched.

Rearing difficulties

32. The major difficulty encountered in rearing L. leucogaster is that

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* ARS Form 442 File No. Biological Shipment Record - Quarantine Facility.

of providing a continuous supply of milfoil flowers. Flower spikes that had just emerged and still had pinkish stigmas and unswollen ovaries were suitable for collection as were submersed buds. Spikes in full flower matured too rapidly to allow much larval development. It was necessary to collect a short section of stem along with the flower spike because the stem provided support for the spike to stand erect and also provided a pupation site for the L. leucogaster larva. If a spike had only a few centimeters of stem it lay on the surface. Flower development was slowed by the cool temperatures in the growth chambers but was not stopped. This provided flower spikes for about 1-2 weeks. Without cooling they would have been suitable for only a couple of days.

33. The large greenhouse cage (Figures 4, 5) required the least amount of daily labor; but because of the continual addition of new flower spikes and the breakdown of the old ones, it was necessary to remove the old material periodically and to observe the stems for pupae. This required at least one entire person-day, but if the stems were not removed, many pupae died. Continuous flooding of the cage improved the quality of the plants and the pupal survival.

34. Diseases were a constant problem, especially on the flowers. Fungus developed quickly because of the high humidity in all the rearing containers or the cage. Fungus was especially heavy in the greenhouse cage by the end of the season; however, no fungicides were able to be used. The stems and flower stalks would break down within a day if the container had been used several times without cleaning with bleach. The common entomopathogenic fungus, Beauveria bassiana (Balsamo) Vuillemin (Figure 6) was always present but never epidemic. It also increased in adundance in the cage near the end of the season. Two additional fungi,

Paecilomyces javanicus and Achyla sp., were isolated from L. leucogaster adults by Mr. Thomas but were not epidemic.

35. As many as 61 pupae were recovered from a rearing jar in which five females were confined with flower spikes for three days. However, usually the pupal yield was less than this and highly variable. A test comparing techniques for rearing larvae in the jars was conducted but the results were lower than normal in all the jars. It has been included as Appendix C.

Biology and behavior

36. Adult description. The adult L. leucogaster is small, about 2.5-3.0 mm, and is covered dorsally with gray-grayish brown scales except for an elongate patch of white scales along the midline of the elytra near the thorax (Figure 7). Yellow or white scales cover the rest of the body. These scales are hydrophobic and a layer of air is trapped around the body when the weevil submerges. The antennae and legs are reddish brown. The tarsi and knees are black. The pronotum has a pair of acute lateral tubercles near the posterior margin which are directly in front of two distinct ridges (fifth strial intervals) on the basal half of the elytra. These tubercles and the ridge on each elytron help separate this species from others. The sexes can be distinguished by the first two abdominal sternites which are convex in the female but slightly flattened and concave along the midline in the male. The measurements of 10 field-collected adults of each sex were. males, length $\bar{x}$ = 2.64$\pm$0.09 mm (r = 2.48-2.80), width $\bar{x}$ = 1.43$\pm$0.06 mm (r = 1.36-1.52); females, length $\bar{x}$ = 2.79$\pm$0.11 m (r = 2.64-3.00), width $\bar{x}$ = 1.48$\pm$0.07 mm (r = 1.40-1.56).

37. Mating. The adults which had been collected in California in

April did not mate until they had been in the laboratory several weeks. Mating was immediate, however, with those collected from the shore in late May-early June. They mated repeatedly and mating pairs were usually observed on the flower spikes or sides of the containers rather than under the water. In order to determine if coupling would take place underwater, five pairs were confined in vials underwater and observed for two hours. This was repeated twice with milfoil stems in the vials and once with milfoil flowers. One coupling was observed in the 10 vials with stems and three couplings in the five vials with flowers. These couplings were of short duration with one lasting 7 minutes and the others 2-4 minutes. Roudier (1957) observed two pairs mating in a glass jar in France and one of these pairs was underwater although he did not observe the actual coupling. Our observations indicate that the flower spikes are the usual mating sites although mating can occur underwater and that mating probably also occurs on shore before the hibernating beetles disperse in search of milfoil flower spikes.

38. Escape and swimming behavior. When adults on the flower spike were threatened, they moved around it so that they could not be seen. If the threat continued, they usually dropped from the plant although they also flew in the large greenhouse cage. In nature flight might be more common than it was in confinement. When they dropped on a milfoil stem, they quickly crawled into the water along the stem. If they dropped on the surface, they swam along the surface until they contacted something. Their swimming resembled the human breaststroke. The forelegs were extended directly in front until they almost touched and the mid- and hindlegs

were extended backwards with a slight separation between them. The forelegs were then swung in a 90° arc to the side providing forward propulsion and the mid- and hindlegs were brought forward to the side. While the forelegs were being extended forward again, the mid- and hindlegs were moved backwards providing the propulsion. The forelegs moved in about a 90° arc, the midlegs in about a 45° arc, and the hindlegs in about a 30° arc. The mid- and hindlegs were moved in unison but the hindlegs did not appear to provide much propulsion. Sometimes the hindlegs were barely moved without an apparent effect on the movement. Forward propulsion thus appeared to be produced mostly by an alternating of a foreleg stroke-midleg stroke-foreleg stroke.

39. Submergence. When the adults crawl into the water, their bodies are surrounded by a thin silvery layer of air which provides them with oxygen (plastron respiration). This air layer allows them to remain submerged for many hours. In the submergence test comparing presence and absence of milfoil in boiled water, one was moribund in each group after 5 hrs, four with milfoil and three without were moribund sometime between 8-25 hrs, and the last one without milfoil was moribund at 28 hrs, although there was a small air bubble in his vial which may have helped prolong his life. In the submergence test comparing river and tap water without milfoil, nine of 10 beetles were moribund in each group sometime between initiation and 15.5 hrs. The last one in river water was moribund at 18.5 hrs and that in the tap water at 24.5 hrs. It appears from the results of these two experiments that the majority of adults do not become moribund until sometime between 8-15.5 hrs of submergence and that some individuals are

able to survive for a complete day. These survival times may be even longer since later observations revealed that some moribund beetles recovered when exposed to air. They thus become moribund, or non-moving, before they are actually dead. This ability to survive long periods of submergence indicates that they should be able to survive in an area like Crystal River where most of the flower spikes are covered at high tide or in a prolonged storm when waves wash over the flowers.

40. Feeding. In laboratory rearing jars, submersed adults fed on both leaves and stems but mostly on the stems just beneath the water's surface. However, most activity and feeding was on the emersed flower spike. All portions of the flower spike were eaten. An entire female flower might be eaten or only one of the four ovaries. Shallow cavities of various sizes were eaten into the stem and with heavy feeding it was completely girdled and destroyed so that the flower spike fell over (Figures 8, 9, 10). Developing seeds were eaten but not after the seed coat had hardened so that it resisted crushing with dissecting forceps. It appeared that the young female flowers and the male flower buds were preferred. When open male flowers were eaten, the weevil's frass was a hardened mass of pollen. Although much pollen was eaten, it was not necessary for oogenesis since females produced eggs even when fed only flower spikes from which the male flowers had been removed. Feeding occurred during both day and night. The total numbers of female and male flowers eaten during their lifetime were counted for seven pairs of weevils in the fecundity test. The largest totals for a couple were 705 female flowers plus 249 male flowers. The largest average number

of flowers eaten daily was 15.3 female flowers and 4.4 male flowers.

41. Oviposition and fecundity. Newly emerged females began ovipositing within 3 days. The most common oviposition site was in the side of an excavated ovary (Figure 11). Eggs were also often placed in an excavation in the ovary at the center of the four stigmas, among or inside the flower buds, among the anthers of open male flowers, in shallow excavations in the stem of the flower spike, and in submersed flower and stem buds.

42. In order to determine the total number of eggs that a female would lay in her lifetime, or the fecundity, 10 females were tested in the rearing room and four in the greenhouse. The average number of eggs and the average longevity for the rearing room females was $\bar{x} = 405.1 \pm 226.4$ eggs/female (r = 145-728) and 44.0 ± 19.7 days (r = 16-61). Three of the females died prematurely between 16 and 19 days after the experiment began. If the data from these three females are not used, the averages become $\bar{x} = 514.3 \pm 174.7$ eggs/female (r= 253-728) and 55.3 ± 9.6 days (r = 44-61). The results for the four greenhouse females were $\bar{x} = 548.8 \pm 308.9$ eggs/female (r = 143-864) and $\bar{x} = 68.8 \pm 32.1$ days (r = 21-90). If the data from the one prematurely dying female are not used, these averages become $\bar{x} = 684.0 \pm 182.6$ eggs/female (r = 499-864) and $\bar{x} = 84.7 \pm 4.7$ days (r = 81-90). The females held in the greenhouse produced more eggs and lived longer possibly because of higher temperatures or the effect of natural lighting. The results of both experiments probably exceed those which would be found in a natural population where longevity should be less and where the females would have to search for flower spikes. However, they do represent a potential

fecundity and longevity which some individuals should attain in nature.

43. Eggs were occasionally removed from the flower spikes and held to determine the number that hatched, or the egg viability. The viability ranged from 35-78% and was similar throughout the duration of the experiment. The relatively low viability was probably due mostly to handling damage.

44. Eggs. The newly deposited ovate egg was pale yellow and was covered with a thin transparent reticulated exochorion. This exochorion turned brownish and hardened if the egg dried, and it probably protects the egg from desiccation. As the egg swelled with the developing larva, the exochorion was split open. The mature egg was colorless and the larva was visible. Eggs measured shortly before larval emergence when the larval head capsule was brown were $\bar{x}$ = 0.53$\pm$0.04 mm (r = 0.45-0.58, n = 22) long and $\bar{x}$ = 0.43$\pm$0.05 mm (r = 0.31-0.48) wide. The duration of the egg stage was 3-4 days at a constant 24°C and 4-5 days at 27°C day and 13°C night with a 16L:8D photoperiod.

45. Larvae. There are three larval stages, or instars. The first instar larva was pale yellow, or cream colored, with a brown head capsule and reddish-brown mandible tips (Figure 12). There were two small dark brown eye spots on each side of the head capsule above the base of the mandibles and the labial palps were dark. The body was slightly flattened dorso-ventrally and the central portions of the segments projectd laterally so that when viewed from above the margins of the body were undulate. There was a row of about 0.04-mm-long setae along each side and two parallel rows along the dorsum. Minute sclerotized plates, or asperites, covered the

dorsum and part of the venter. There were, of course, no legs or prolegs, but there were two extrusile posterior projections on the last segment which aided locomotion. Newly emerged, or neonate, larvae were about 1.0-1.2 mm long and older ones prior to molting were about 1.8 mm long. The head capsule width was $\bar{x} = 0.24 \pm 0$ mm (n = 10). The duration of this stadium was 2 days at 24-27°C.

46. The second instar larva was similar to the first. It could be differentiated, however, by a larger head capsule which was $\bar{x} = 0.34 \pm 0.01$ mm (r = 0.32-0.34, n = 10) wide. The body length, depending upon age, was about 2.4-3.7 mm. The duration of this stadium was also 2 days at 24-27°C.

47. The third instar larvae (Figure 13) was relatively more flattened than the other two and the lateral projections were more obvious. The body was darker and varied from reddish yellow to deep pink. The head capsule and prothoracic shield were dark brown. The asperites were larger and more noticeable and could be seen with a 10x hand lens. The tracheae were obvious and the elliptical spiracles were large, about 0.04 mm wide. The head capsule width was $\bar{x} = 0.53 \pm 0.02$ mm (r = 0.50-0.56, n = 7) and the length varied from about 4.2-6.0 mm, again depending upon age. The duration of this stadium prior to initiation of a cocoon was 3-4 days at 24-27°C.

48. The total duration of the three instars of the active larval stage was 7-8 days at 24-27°C, 8-10 days at a constant 24°C, and 9-11 days at 27°C day and 13°C night with a 16L:8D photoperiod.

49. Neonate larvae fed mostly inside the ovaries or flower buds, but as the first instar larvae grew, they began to feed externally on the flowers

as did the two latter instars. The large third instar larvae encircled the stem while feeding on the ovaries, developing seeds, and stem. If one flower spike was not sufficient for maturation, the larvae crawled along the submersed stems to other flower spikes or occasionally fed on submersed stems or flowers. When flower spikes with mature larvae were pulled underwater and held there, the larvae usually moved upwards to the emersed portion or if there was none, they remained underwater and fed. Six larvae remained on submersed flowers from 6-19 hrs after which they either entered the air-filled stem to feed or pupate or died. They were thus able to survive submergence for a period comparable to that of the adults, but in addition they were able to escape into the stem. Young larvae were not tested, but when confined in closed plastic vials with flower spikes, they escaped high humidity by entering the stem and would presumably do likewise when submersed. Larvae that fell on the water's surface greatly extended their body segments and swam with a serpentine motion. The lateral projections may have aided this by providing additional forward push. This might be the reason why these projections are so strongly developed in this species.

50. Cocoon and pupa. The mature larva excavated a hole in the submersed stem and formed a brown ovate cocoon. The cocoon was formed at various distances beneath the flower spike but mostly about 3-13 cm beneath it. This cocoon was half in the stem and half out (Figure 14). The outer half was leathery and thicker than the inner half which had holes that allowed air to enter from the stem. Without this air the pupa died which happened when the stems became waterlogged. Pupae removed from the submersed

cocoon to moist cotton developed normally. Shortly after completing the cocoon, the larva became immobile and formed a prepupa with a contracted abdomen and defined thoracic regions. The duration from the closing of the cocoon to the actual pupal formation was 3 days at 24-27°C.

51. The head and thorax of the newly formed pupa were white and the abdomen was light pink (Figure 15). Numerous setae were prominent on the head and the dorsum. The female pupa was larger than that of the male. The measurements of both sexes were: male, length $\bar{x}$ = 1.38±0.04 mm (r = 1.32-1.46, n = 10), width $\bar{x}$ = 0.77±0.03 mm (r = 0.74-0.82); females, length $\bar{x}$ = 1.46±0.06 mm (r = 1.40-1.58, n = 10), width $\bar{x}$ = 0.82±0.03 mm (r = 0.78-0.88). This stadium lasted 2-4 days but mostly 3 days at 24-27°C.

52. The white teneral adults remained in the cocoons for 1-3 days while they hardened and darkened. They were still not completely hardened when they emerged by chewing holes through the cocoons, but they had hardened sufficiently to function. The integument of the new adult was actually yellowish and it was the scales which were all snowy white.

53. Weevil and flower development. Although the weevil has a relatively short larval developmental period, the milfoil flower spike also has a short developmental period. In order to determine how long the flower spikes would be emersed and thus available to the larvae, nine flower spikes in an outdoor pool were observed from the time that the buds broke the surface until all of the female whorls had submerged. The average number of female whorls was 5.6 (r = 4-7) and that of the male whorls was 3.6 (r = 2-5). Two flower spikes had no male flowers. The female flowers were open after 1.8 days (r = 1-2) and the first male flowes were open 3.6 days (r = 2-5) after the first female flowers. The female

whorls on a spike opened simultaneously but the male whorls opened 1-2 per day. The first whorl of female flowers sank an average 4.4 days (r = 1-8) after the buds had emerged and the last whorl of female flowers sank after 8.6 days (r = 7-11). Male flowers were still exposed for about two more days but by that time they were dry and would probably not be acceptable to the larvae.

54. The 8.6 days that at least one whorl of female flowers was emersed corresponds closely to the 7-8 days duration of the active larval stage at 24-27°C. However, in order for a larva to mature on one flower spike at these durations, the egg would have to be deposited in the submersed bud and the neonate would have to hatch as the bud emerged. Females did indeed oviposit in submersed buds but when given a choice they oviposited more in emersed flowers. In nature, the day temperatures near the mat might be higher than the 24-27°C in these tests, and thus larval development might be faster. In addition, the average number of female whorls might be greater or they might be emersed longer. Whether this is true or not, the development of L. leucogaster is remarkably well adapted to its host suggesting a long relationship with it.

55. The duration of the life cycle of L. leucogaster from egg to egg was 19-25 days. There should thus be multiple generations in nature instead of a single generation as reported by Scherf (1964) and Dieckmann (1972). In the northern U.S. there would probably be two or three generations per year; in Florida as many as five generations might be possible since the plants flower continuously from June-November.

Host specificity

56. Adult feeding. Since L. leucogaster is native and is already

distributed over much of the U.S. (Figure 16), the host-specificity tests were not as extensive as would be necessary for an exotic insect species. The two collection localities in Georgia surely represent range extensions and suggest that this species might eventually extend its range to other areas where milfoil has been introduced.

57. The results of the adult no-choice feeding tests are listed in Table 1. The only plant species which was eaten in amounts similar to those on milfoil was Pennsylvania smartweed. Most feeding on this species was on the flower buds and petals although occasionally submersed leaves were eaten. The longevity of the adults on this species equaled or exceeded that on milfoil. Interestingly, dotted smartweed was not eaten. The flowers and buds of two other smartweeds, mild smartweed (P. hydropiperoides Michx.) and hairy smartweed (P. hirsutum Walt.), were also eaten in the nonreplicated miscellaneous tests. Baloch et al. (1972) reported that the Phytobius sp. from Bangladesh, whose hosts were two species of watermilfoil, fed on only one of the two smartweed species that they tested. The adults of this weevil remained on smartweed when watermilfoils were not flowering, but they did not breed on the smartweed.

58. Feeding on parrotfeather and the two mermaidweeds was about 1/4-1/3 that on milfoil. The weevils survived as well on parrotfeather but not as well on mermaidweeds as on milfoil. Only nibbling (minor feeding) occurred on broadleaf watermilfoil flower spikes but the spikes were not in good condition.

59. No feeding or only minor feeding occurred on the four species in the Onagraceae and Lythraceae which are usually placed in the same order as

Haloragaceae. The flower petals of one replicate of crape myrtle were eaten but this was not surprising since petals are generally thought to lack anti-feeding substances. In fact, the petals of several plant species were initially fed upon in the nonreplicated miscellaneous tests but the feeding was not sustained. These were an orchid, Galactia sp., a composite, Erigeron sp., and Rhexia sp. (Melostomaceae).

60. The feeding on rhubarb and beet leaves was also not surprising since other species in the families Polygonaceae and Chenopodiaceae are hosts of the weevil relatives of L. leucogaster and since these two plant families might be related to milfoil. Lawrence (1951) illustrates a dendrogram for the phylogeny of angiosperms based upon Hutchinson's classification in which the Lythrales, Polygonales, and Chenopodiales are derived from a common ancestor. Milfoil is in the Lythrales. The dendrograms based upon the classifications of Hallier, Bessey, and Takhtajan, as illustrated by Lawrence (1951), place the Polygonales and Chenopodiales far removed from the Lythrales. The weevils apparently support Hutchinson's views. The leaves of the beet seedlings were toxic, causing 80% mortality within four days.

61. The minor feeding on the pondweeds was initial feeding on the submersed leaves and stems but this feeding was of short duration. The flower spike which was superficially similar to that of milfoil sustained only occasional nibbling. After 25 days there was 100% mortality on both pondweeds, but only 13% on milfoil.

62. Oogenesis. Except for single eggs deposited by the field-collected weevils during the first ten days on mermaidweed (P. pectinata)

and watercress, oviposition in the feeding tests occurred only on Pennsylvania smartweed, parrotfeather, and milfoil. Since the weevils used in these tests had fed previously on milfoil, oogenesis (formation of eggs) tests were initiated with newly emerged females that had not yet eaten. Pennsylvania smartweed was tested in a Type 1 test in plastic vials. At various intervals after oviposition commenced on milfoil, a female on smartweed was dissected. No egg development was found in the 10 females even though the last female was not dissected until after 1-½ months. Parrotfeather and mermaidweed (P. pectinata) were also tested in Type 1 tests. The 10 females on mermaidweed were dissected like those on smartweed but no eggs developed. The females on parrotfeather produced $\bar{x}$ = 86.4±90.9 eggs/female (r = 3-255, n = 10), and lived $\bar{x}$ = 31.4±11.9 days (r = 12-42). Although this was much less than the 405 eggs/female and the 44 days obtained with insects on milfoil, it indicates that parrotfeather is a potential host plant. Most of the eggs did not hatch, but this may have been influenced by the early removal of the males.

63. Larval survival. Larvae were able to develop only on parrotfeather in addition to milfoil, and even on parrotfeather development was marginal. In 1978 a total of 40 larvae were placed on parrotfeather in vials but none lived more than 2-3 days. Seventy-five of the 1978 field-collected adults were confined in an aquarium with parrotfeather until most of them died and no larvae developed even though eggs were deposited. The eggs from the females on parrotfeather in the oogenesis tests were placed on parrotfeather in the greenhouse cage in a Type 6 test. Three cocoons were produced. Additional Type 1 larval tests were negative for creeping

waterprimrose (15 larvae), mermaidweed (P. pectinata) (1[illegible] larvae), watercress (15 larvae), Illinois pondweed (7 larvae), and Pennsylvania smartweed (10 larvae). Most of the other plant species were tested with small numbers of neonate larvae in Types 1-5 tests; but since the milfoil controls rotted and the control larvae died, these results had no control. However, all were negative.

64. Future testing. L. leucogaster is a good candidate for introduction into countries where it is not native. If other genera of Haloragaceae are of importance, they should be tested although their growth habits are apparently not similar to those of milfoil. Even though other species of watermilfoils might be attacked, they could probably maintain natural, non-weedy, densities even with reduced seed production. In a tropical country without a cold winter to force the weevils to hibernate, important plants in the Polygonales and Chenopodiales should be tested since adults might feed on these when milfoil was not present.

Release at Crystal River, Florida

65. Adults and eggs of laboratory-reared L. leucogaster were released at and near Bagley Cove, Crystal River, Florida, on 22 August 1979. Mr. Russell Theriot, APCRP, WES, Dr. Joseph Balciunas, Aquatic Plant Research Center, University of Florida, Ft. Lauderdale, and the authors placed 187 mixed adults of approximately equal sexes at two locations along the inner edge of the milfoil mat at Bagley Cove (Figure 17). A small mound was formed by pulling the submersed stems above the surface and the chilled adults were taken from a cooler and placed onto this mound. These stems along with paper towels from the jar provided resting spots for the weevils.

When weevils are placed directly on the water surface, they fly immediately. Ten flower spikes with at least four eggs per stalk were placed among stems in a mat at the mouth of a small channel entering the river west of Bagley Cove. There were few erect flower spikes but many buds in the mat. It was sunny and hot when the adults were released about midday but overcast and beginning to rain when the eggs were released at 1400. It rained heavily immediately afterwards. Low tide was at 1029 which provided a maximum exposure of the plants during the beetles' adjustment period. Most flower stalks were covered during high tides.

66. A female L. leucogaster was collected along with adults of another native weevil, Perenthis vestitus Dietz, on 6 September 1979, about 100 m southeast of the release point. The mat where it was collected was connected with that at Bagley Cove by a narrow strip of flowering plants near the shore. No further specimens were collected until November even though large collections of P. vestitus adults and flower stalks were made almost weekly. On 8 November two male L. leucogaster were collected along with P. vestitus adults in a large canal which enters the river west of Bagley Cove. This area is about 1.5 km overland from the release site and about 2.6 km by water. Although these may have been released males, they were probably the progeny of the released beetles.

Differentiation of the milfoil weevils

67. L. leucogaster, E. velatus, and P. vestitus all live on milfoil throughout much of the U.S. (Figure 18). The sharply pointed prothoracic tubercles and the raised elytral ridges separate L. litodactylus from the other two species which have small, not strongly pointed tubercles and

elytral ridges of equal heights. E. velatus can be distinguished from P. vestitus by the long setae on the basal tarsal segments of E. velatus. E. velatus is also readily distinguished by the yellowish stripe along the entire midline of the elytra.

68. The larvae of L. leucogaster and E. velatus can be differentiated from those of P. vestitus by the asperites, or minute dark spots, which are on the skin of the first two species. They were noticeable with a 10x hand lens on L. leucogaster but were more difficult to see on E. velatus. The living larva of L. leucogaster was usually pink compared to light yellow for P. vestitus. Viedma (1970) described the larva of E. velatus as whitish yellow, while Scherf (1964) described it as yellowish-green.

69. The cocoon of L. leucogaster projects out of the stem so that it appears hemispherical. The cocoon of P. vestitus is entirely inside the stem and is visible only through the small circular larval entrance hole. According to both Scherf (1964) and Schwarz (1887), the cocoon of E. velatus is spherical and is attached externally to the stem or leaves.

70. Except for the size, the eggs of L. leucogaster and P. vestitus are difficult to distinguish with certainty. However, generally those of P. vestitus are covered by excrement while those of L. leucogaster are not. P. vestitus also places a larger percentage of eggs in shallow excavations in the stem of the spike than does L. leucogaster which prefers the flowers. Scherf (1964) indicates that E. velatus oviposits externally on the leaves rather than on the flower spikes. When the first two species oviposited on submersed leaves, they did so in the leaf buds. (Competition between L. leucogaster and P. vestitus is discussed in Appendix D.)

Studies with Acentria nivea

Field observations

71. The June 19 field collection yielded additional information to that already reported by Batra (1977) who made studies in 1975-1976 at the same locality. The surface water temperature was 17°C and most northern watermilfoil plants had not yet started to grow though a few in shallow water had bright green tips of new growth. The majority were rooted, upright, perennial plants with multiple shoots, about 13-50 cm tall, not broken shoots lying on the bottom. Such plants were generally about 30-100 cm apart and were grouped into small clusters which were separated from other clusters by weed-free areas. There were also scattered single plants and some larger more densely populated patches, especially among large boulders that were grouped in several places on the otherwise flat silty bottom. Most plants were covered with filamentous algae and other debris and some of the broken shoots lying on the bottom were so covered with algae that they looked like pure algal masses. Plants were collected at depths of about 1-3 m and mostly at 1.5-2.0 m. The clumped distribution of the perennial plants and their upright stature makes it difficult to survey them when they can not be seen from the surface. Batra (1977), who collected in the same area from a boat with a rake, concluded that the plant population overwintered as broken shoots or propagules that had sprouted by June, but in fact, only a minority of the plants overwintered that way.

72. Although northern watermilfoil was the dominant species, there were small areas where coontail was abundant. A few scattered plants of waterweed (Elodea canadensis Michx.) were also collected.

73. Almost all watermilfoil plants had one or more larval cases of A. nivea attached. The cases were also found on coontail and a few on waterweed. However, the waterweed had been with milfoil overnight, before the plants were examined, so there was a possibility that the larvae had transferred. The majority of larvae were large (10-13 mm), but smaller larvae (5-7 mm) were also present.

74. A population of active adults was found at a shallow inlet behind Cabins 14 and 15 in Robert Moses State Park, near Massena, New York, on the nights of June 21-22. This is a month earlier than adults were reported by Batra (1977) at the deeper Barnhart Marina location. The inlet was about 50 m long and 6 m wide, and the maximum water depth fluctuated from about 0.3 to 1.0 m due to a tidal-like current that was probably produced by the nearby river locks. (The current changed directions about every minute as it flowed in and out of the inlet.) Small clumps of non-flowering milfoil were scattered along the inlet. From 2200-2400 on both nights, males were observed flying in wide circles just above the surface of the water, which they appeared to touch occasionally. Since they flew so close to the surface they could not be collected with an aerial net except by submersing it and then raising it slightly as they passed over. When caught in this way, they did not attempt to fly out of the open net but kept flying around the edge. They were easily transferred from the net if a cup was dipped into the water directly behind them as they were flying which apparently caused them to

be sucked into the cup with the water. They did not fly out of the cup and followed the water when it was transferred to a holding container. They were easily handled in this manner, even in the laboratory. Both nights males were observed sitting on emergent vegetation and algal mats, but no females could be found even when the plants were illuminated from below by a submersible lamp. No males were collected in or under black-light traps placed near the shore and about 75 m away at the cabin even though Treat (1954, 1955) reported that they came to both blacklights and incandescent lights. The night of June 22, the air temperature near the water surface was 14°C at 2300, and the water temperature was 19.4°C. The temperature on June 21 was not noted, but it was a warmer night. However, since Treat (1954) collected adults at lights on nights when temperatures were 14.5 and 15°C, our June temperature should not have been too cold. Perhaps the attraction of the moths to light is associated with the appearance of winged females since whenever more than a few specimens have been collected at lights both sexes have been present. It is possible that a "mating swarm" is formed and migration occurs when the population density becomes high and winged females are produced. The winged females may be recessive homozygotes resulting from continued inbreeding of the flightless females. A mating flight might thus be a means of outbreeding from the numerous "population islands"; then for several generations after a mating flight, the females would be again flightless. In fact, the females collected at lights by Treat (1955) readily laid fertile eggs, which produced flightless females. A swarm formation was mentioned by Nigmann (1908) who quoted J. P. Barrett as stating that A. nivea occurs in swarms.

Nigmann doubted this and considered the swarms merely large emergences at those host plants receiving egg batches. We obtained only flightless females during our rearing. Batra (1977) had a similar experience when she reared larvae collected in 1975 and 1976 from the same location. However, she reared one winged female and several flightless females from larvae collected in autumn 1978 (personal communication).

Distribution

75. The distribution of A. nivea is illustrated in Figure 19, and suggests that it is indeed an introduced species. The distribution correlates closely with that of milfoil. The 1927 discovery date predates the first records of milfoil in the St. Lawrence River system as reported by Reed (1977), but since milfoil is often confused with northern water-milfoil there is an excellent chance that milfoil was in the system long before its presence was confirmed. The lack of A. nivea collection records before 1927 also suggests that it was introduced. The small size and aquatic behavior certainly did not prevent it from being a well studied species in Europe and should not have prevented its early discovery here if it were native. Exact collection localities obtained from the literature and from personal communications are listed in Appendix B.

Biology and rearing

76. The male A. nivea was a small (12 mm), winged, grayish-white moth, (Figure 20), but the female had reduced wings and was flightless (Figure 21). The larvae (Figures 22, 23) did not have gills as do the larvae of the more common genus Parapoynx (Figure 24). The young A. nivea larvae were almost transparent and were difficult to detect without magnification. The gut contents and tracheae of the older larvae were visible

through the body wall but the larvae were not as transparent as the young ones and were easier to see. They also differed from most Parapoynx larvae by the type of shelter they built. A. nivea usually tied together several leaves of milfoil to form a stationary shelter (Figure 25) from which it fed or from which it exited to feed and new shelters were often produced. They were also often found in the leaf buds which formed natural shelters. Most Parapoynx larvae cut leaves from their host plants to form cases that they carry with them and from which they feed. Both Parapoynx and A. nivea larvae can also be found feeding in the stems of their hosts. Larvae of the polyphagous Synclita obliteralis (Walker) occasionally fed on milfoil in outdoor pools. It was similar to A. nivea in lacking gills but it lived in an air filled case and had a characteristic dull white skin (Figure 26) compared to the transparent skin of A. nivea. The mature A. nivea larva excavated one side of the stem and formed a tightly woven white elongate cocoon (Figure 27) which was filled with air from the damaged stem. When the stems became waterlogged, the pupae died.

77. Most females in the laboratory emerged at night or in late afternoon. They rested on the water's surface and at night they lifted their abdomens in the air to attract the flying males. Mating was not observed. Females generally died before the second night after emergence but if they did not they were very weak. When females were disturbed they swam rapidly on the surface with aid of specially adapted middle and hind legs. Disturbed females attempted to submerge, but since the body scales are apparently hydrophobic, they were unable to submerge unless

holding onto stems. One male was observed crawling rapidly along a stem into the water in a holding jar during the field collections, but this observation was not repeated during the laboratory rearing. The male was removed immediately to check the sex, so that the length of time it could remain submerged was not determined. Berg (1942) cited the only other report of males submerging and he questioned the normality of this behavior. However, it might be an avoidance response to predators, especially to bats. Females were observed clinging to underwater stems (Figure 28) where they oviposited single clusters of yellowish ovate eggs. Eggs were also found on styrofoam floats (Figure 29).

78. Larvae emerged from only one of the laboratory egg batches that were separated and observed. The first neonate larvae along with the unhatched eggs from this batch were placed immediately in a jar filled with a test plant so that initial larval development was never closely observed. However, Berg (1942) reported that larvae hatched after about 12-13 days and that initially they bored into the stems of the host plants. Later they left the stems and formed shelters. Berg detailed the biology and developmental times and illustrated the swimming legs, genitalia, mouthparts, and various stages.

79. Nobody has previously reported rearing A. nivea past the emergence of adults from field-collected eggs or larvae. One reason is that the long developmental time results in staggered emergence; only single or a few adults emerge on the same night and they die or are weak by the second night. From the adults collected as small larvae in September 1978, we obtained at least three generations by January 1980. Attempts to monitor the colony to

obtain fertile eggs for experiments were unsuccessful except in one case. Larvae were produced only in containers that were left undisturbed. Many of the large field-collected larvae from the June collection crawled out of jars that were held in sleeve cages and spun cocoons among paper toweling on the cage floors. None of these survived. Nigmann (1908) also reported that larvae crawled out of the rearing containers, and Treat (1955) mentioned that two larvae, which eventually died, made cocoons under the lid of a holding jar. Initially it was thought that this behavior was a response to low dissolved oxygen but it continued even when the jars were aerated. Therefore it was probably aviodance of high temperatures since the jars were then being held above 24°C, which subsequent observations indicated was too high. The larvae withstood high temperatures (at least 40°C) for short periods during equipment breakdowns, but prolonged exposure above about 22°C retarded development or lead to death. In nature larvae can escape high surface temperatures by moving deeper into the water. Larvae were not observed crawling from containers after we began holding them below 22°C.

80. Low dissolved oxygen, however, was also found to be important. During transport of field-collected larvae in plastic bags densely packed with plant material, larvae became immobile within a few hours if the bags were not exposed to light. Most of these immobile larvae recovered when the bags were aerated during and after the trip to the laboratory. During long dark periods, such as shipping, oxygen would be critical; it would be necessary to either provide oxygen or to include only a small number of plants in a large volume of water. In normal laboratory rearing procedures, dissolved oxygen was not critical.

81. A fungus, Achyla sp., was isolated from dead larvae in our colony by Mr. Gerard Thomas, but it was probably saprophytic.

Host specificity

82. The no-choice test confirmed the reports of other authors that A. nivea feeds on a variety of plants. The positive results of this test and the observations of others are summarized in Table 2. Moderate to heavy feeding was observed in this test on hydrilla, parrotfeather, milfoil, southern naiad, Illinois pondweed, and both mermaidweeds. Southern naiad and Illinois pondweed were especially damaged. Only minor feeding occurred on creeping water primrose, watercress, and fanwort, though the latter species has a growth form and leaf structure similar to those of milfoil. There was no feeding on alligatorweed, waterpennywort, frogbit, dwarf arrowhead, cattail, and slender spikerush, though relatives of this latter species are hosts of other shoenobiine moths. Salvinia leaves were not eaten, but the roots were always severed. Although the larvae in this test fed on parrotfeather, in Batra's (1977) they did not. Perhaps this is because the broader aerial leaves were submersed and tested in this test since only they were available.

83. Large larvae given a choice of coontail, hydrilla, slender naiad, Illinois pondweed, and milfoil fed and made cases on all of them. Small larvae confined with only one plant species developed and made cocoons on Illinois pondweed, hydrilla, coontail, Brazilian elodea, and milfoil. The cocoons were then placed in one container to obtain mated females. An egg batch with emerging larvae was placed on Illinois pondweed and medium-sized larvae were obtained. This result combined with the preceding one leaves

little doubt that the literature reports that pondweeds are host plants are valid (Berg, 1942; Gaevskaya, 1969; Nigmann, 1908). Possibly not all of the species listed in Table 2 are true or even potential host plants, but they do indicate a broad feeding range and at least a varied host range since eight plant families are represented.

PART IV: CONCLUSIONS

Studies with Litodactylus leucogaster

84. The specialized biology of L. leucogaster indicates that it is well adapted to milfoil. Oviposition was always accompanied by feeding and undoubtedly host plant recognition and acceptance occurred during this feeding, thus preventing indiscriminate oviposition. Most eggs were deposited in ovaries or buds rather than merely externally on the stem. Larval development and flower development required about the same small amount of time and the relatively flattened larva was well adapted for curling around the milfoil flower spike and clinging to it while feeding. The larva, as well as the adult, was able to survive lengthy submergence periods which would be necessary for an insect on milfoil. The submersed cocoon required a hollow air-filled stem with a minimum diameter which was provided by the stem of milfoil. In addition to the specialized biology, the holarctic distribution of the weevil and the plants indicates an ancient relationship with watermilfoils as does the fact that watermilfoils are hosts of at least three closely related weevil genera.

85. In nature L. leucogaster is apparently specific to watermilfoils and probably to only certain species in the genus. The adults could feed and survive long periods on smartweeds, if necessary, but they have not been reported on these plants even though smartweeds have a well-collected insect fauna. The adults could feed for short periods under unforeseen circumstances on plants in the Chenopodiaceae or Polygonaceae; but they could not develop on them and there is no evidence that they

would feed on them in nature. If L. leucogaster were of interest to countries in the Southern Hemisphere where there are other non-tested genera of Haloragaceae, it would be advisable to test species in these genera.

86. Adults and larvae of L. leucogaster can cause extensive damage to milfoil flower spikes under artificial conditions, but whether they can do the same under field conditions remains to be seen. The high density of flower spikes in a heavy milfoil mat and the short period that the flowers on those spikes are available to the insects would moderate the damage in a natural situation. In the absence of data both on the role that seeds play in the population dynamics of milfoil and on the potential damage by L. leucogaster, it is not possible to accurately assess the importance of L. leucogaster as a potential control agent. However, reduction in the total seeds produced by the milfoil mat should be preferable to no reduction, if the costs to obtain the reduction are not high. If an effective disease is found in the future that is able to invade the host plant more easily through the weevil feeding damage, then the importance of the weevils would increase and higher costs might be acceptable. Obviously biocontrol agents which cause greater damage and stress to the plants will be needed for control of milfoil, but a complex of agents, including seed feeders, should be best.

87. The distribution of L. leucogaster as seen in Figure 16 indicates that it is widespread on northern watermilfoil but that it may not be present at many of the scattered milfoil localities throughout the South or in the principal milfoil areas in the Northeast. The small size

and aquatic behavior has undoubtedly prevented its discovery at additional locations. The distribution also indicates that the species can survive varied climates, for example those in Central California, Canada, and southern Georgia. Thus it would probably be able to establish at most of the milfoil locations.

Studies with Acentria nivea

88. The various host records reported in the literature and the results of the laboratory tests leave little doubt that A. nivea is not specific to milfoil and that it has a relatively wide feeding range. Since it already occurs in the U.S. and is apparently increasing its range, it may eventually arrive at most milfoil locations. Whether it should be introduced for biocontrol of milfoil prior to the natural arrival will need to be decided by the individual states. The long developmental period and the limited mobility of the flightless females of A. nivea should limit the buildup of large populations to host plants that are themselves at a high density. At high densities even the most beneficial native plant species are usually considered nuisances so that damage by A. nivea would be acceptable. If a dense stand of a species such as pondweed or naiad provided food for waterfowl and so was desirable, the feeding by the waterfowl would devastate the A. nivea population and prevent it from increasing. If the hypothesis that the winged females are produced at high population densities is correct, then the migration by the winged females would dramatically reduce a high population of A. nivea before it destroyed other plant species after the crash of its

principal host plant. Populations of A. nivea would probably also be limited by natural enemies. Although Nigmann (1908) reported an unidentified parasitic ichneumonid wasp from the pupa and a phorid fly possibly from the pupa, most natural enemies listed by him in Europe were generalized predators such as water bugs, water mites, water beetles, spiders, fish, and bats. He also listed a fungus disease. In addition, Batra (1977) reported planarian egg predators in her U.S. rearings. Since most aquatic communities include large numbers of these generalized predators and also contain diseases, A. nivea populations would be highly vulnerable to natural enemies even though it is an introduced species.

89. The preceding arguments against the possibility of A. nivea becoming a noxious species also apply against it being of great benefit for biological control of milfoil or other host plant species. The observations of Nigmann (1908) and Batra (1977) that the larvae do not feed on algal-covered leaves indicate that a large proportion of a milfoil mat would not be attacked by A. nivea larvae, especially in Florida. The top layer of milfoil stems at Crystal River was heavily covered with algae by at least midsummer. If, however, a complex of agents is desired for control of milfoil, as will probably be necessary, then A. nivea could be considered for that complex, especially since no substantial numbers of native moths have been found or reported on it. For example, only small numbers of Parapoynx allionealis Walker and P. obscuralis (Grote) were found on the plants collected at Crystal River as food for the colony, and only a few P. badiusalis (Walker) larvae were found on milfoil plants in Currituck Sound by Apperson and Axtell (in press). There are no other

reports of lepidopteran larvae on milfoil in the U.S. although there are reports of larvae on other watermilfoils.

90. Larvae of A. nivea fed mostly on milfoil leaves, but they did girdle stems breaking off small fragments. Although these fragments might form new shoots and aid in the spread of the plant as suggested by Batra (1977), the natural spread of this species once it invades a waterway is so efficient that the effect of A. nivea feeding should be of little consequence. Stems are broken by wave action and by man's activity, and there are autofragmentation periods when the stems break apart naturally.

REFERENCES

Aiken, S. G., Newroth, P. R., and Wile, I. 1979. "The Biology of Canadian Weeds. 34. Myriophyllum spicatum L.," Canadian Journal of Plant Science, Vol 59, pp 201-215.

Apperson, C. S. and Axtell, R. C. In Press. "Arthropods Associated with Eurasian Watermilfoil in the Currituck Sound, North Carolina," Journal of the Georgia Entomological Society.

Baloch, G. M., Khan, A. G., and Ghani, M. A. 1972. "Phenology, Biology, and Host-Specificity of Some Stenophagous Insects Attacking Myriophyllum spp. in Pakistan," Hyacinth Control Journal, Vol 10, pp 13-16.

Batra, S. W. T. 1977. "Bionomics of the Aquatic Moth, Acentropus niveus (Oliver), a Potential Biological Control Agent for Eurasian Watermilfoil and Hydrilla," Journal of the New York Entomological Society, Vol 85, pp 143-152.

Berg, K. 1942. "Contributions to the Biology of the Aquatic Moth, Acentropus niveus (Oliv.)," Videnskabelige Meddelelser Dansk Naturhisterisk Forening Kovenhavn, Vol 105, pp 59-139.

Cook, C. D. K. 1974. Water Plants of the World. A Manual for the Identification of the Genera of Freshwater Macrophytes. Dr. W. Junk, ed., The Hague, Netherlands. 561 pp.

Dalla Torre, K. W. and Hustache, A. 1930. "Curculionidae Ceuthorrhynchinae," Vol 28, Part 113, pp 115-135 in S. Schenkling Coleopterorum Catalogus, W. Junk, Berlin, Germany.

Dieckmann, L. 1972. "Beitrage zur Insektenfauna der DDR: Coleoptera-Curculionidae. Ceutorhynchinae," Beitrage zur Entomologie, Vol 22, No. 1/2, pp 3-128.

Gaevskaya, N. S. 1969. The Role of Higher Aquatic Plants in the Nutrition of the Animals of Fresh-Water Basins, Vols 1-3, National Lending Library for Science and Technology, Yorkshire, England. 629 pp.

Habib-ur-Rehman, Mushtaque, M., Baloch, G. M., and Ghani, M. A. 1969. "Preliminary Observations on the Biological Control of Water-Milfoils (Myriophyllum sp. - Haloragidaceae)," Commonwealth Institute of Biological Control Technical Bulletin, No. 11, pp 165-171.

Judd, W. W. 1950. "Acentropus niveus (Oliv.) (Lepidoptera.Pyralidae) on the North Shore of Lake Erie with a Consideration of its Distribution in North America," The Canadian Entomologist, Vol 82, pp 250-252.

Kissinger, D. G. 1964. Curculionidae of America North of Mexico. A Key to the Genera, Taxonomic Publications, S. Lancaster, Massachusetts.

Lawrence, G. H. 1951. Taxonomy of Vascular Plants, 3rd ed., MacMillan Co., New York. 823 pp.

Lekic, M. and Mihajlovic, Lj. 1970. "Entomofauna of Myriophyllum spicatum L. (Halorrhagidaceae), an Aquatic Weed on Yugoslav Territory," Journal for Scientific Agricultural Research, Vol 23, No. 82, pp 59-74, (Translation of Arhiv za Poljoprivredne Nauke, Vol 23, No. 82, pp 63-76.

Leng, Charles W. 1920. Catalogue of the Coleoptera of America, North of Mexico, Cosmos Press, Cambridge, Massachusetts. 470 pp.

Muenscher, W. C. 1944. Aquatic Plants of the United States. Cornell University Press, Ithaca, New York. 374 pp.

Nigmann, M. 1908. "Anatomie und Biologie von Acentropus niveus Oliv.," Zoologische Jahrbucher Abteilung fur Systematik, Vol 26, pp 489-560.

Reed, Clyde F. 1977. "History and Distribution of Eurasian Watermilfoil in United States and Canada." Phytologia, Vol 36, No. 5, pp 417-436.

Ritsema, O. 1878. "Acentropus niveus Oliv. in Zijne Levenswijze en Verschillende Toestanden," Tijdschrift voor Entomologie Gravenhage, Vol 21, pp 81-113.

Roudier, A. 1957. "Localities Nouvelles Francaises ou Espangnoles de Curculionides - Description d'une Sous-Espece Nouvelle - Remarques Diverses," L'Entomologiste, Vol 13, pp 24-36.

Scherf, Heinz. 1964. "Die Entwicklungsstadien der Mitteleuropaischen Curculioniden (Morphologie, Bionimie, Okologie)," Abhandlungen Senckenbergischen Naturforschenden Gesellschaft, No. 506, 335 pp.

Schwarz, E. A. 1887. "On the Genus Phytobius," Proceedings, Entomological Society of Washington, Vol 1, pp 75-76.

_____. 1892. "Note on Phytobius," Proceedings,Entomological Society of Washington, Vol 2, pp 165-167.

Sheppard, A. C. 1945. "A New Record for Canada (Lepidoptera)," Canadian Entomologist, Vol 77, p 55.

Treat, A. E. 1954. "Acentropus niveus in Massachusetts, Remote from Water," The Lepidopterists' News, Vol 8, pp 23-25.

_____. 1955. "Flightless Females of Acentropus niveus Reared from Massachusetts Progenitors," The Lepidopterists' News, Vol 9, pp 69-73.

Viedma, M. G., de. 1970. "Descripcion de la Larva de Eubrychus velatus Beck (Coleoptera, Curculionidae)," Miscelanea Zoologist, Vol 2, No. 5, pp 99-101.

TABLE 1. RESULTS OF NO-CHOICE FEEDING TESTS[a] WITH ADULTS OF *LITODACTYLUS LEUCOGASTER*

PLANT FAMILY Plant Species	Common Name	Type of Tests[b]	Relative Feeding Damage[c]	Total No. of Beetles Tested	Total No. of Replications
HALORAGACEAE					
Myriophyllum spicatum L.	Eurasian watermilfoil	1	+++++	42	8
M. aquaticum (Vell.)Verdc.	parrotfeather	1, 6	++	27	4
M. heterophyllum Michx.	broadleaf watermilfoil	1	+	18	3
Proserpinaca palustris L.	mermaidweed	1	++	12	3
P. pectinata Lam.	mermaidweed	1, 6	++	32	5
ONAGRACEAE					
Ludwigia repens Forst.	creeping waterprimrose	1	0	30	6
LYTHRACEAE					
Rotala ramosior (L.)Koehne	tooth cup	5	0	8	4
Lagerstroemia indica L.	crape myrtle	1, 2, 3	+	62	11
Ammania coccinea Rottle.	purple ammania	5	+	12	6
POLYGONACEAE					
Polygonum punctatum Ell.	dotted smartweed	1	0	12	3
P. pennsylvanicum L.	Pennsylvania smartweed	1	++++	30	5
Rheum rhabarbarum L.	rhubarb	2	++	30	7
CHENOPODIACEAE					
Beta vulgaris L.	beet	4, 5	++	20	5
PORTULACACEAE					
Portulaca grandiflora Hook.	moss rose	5	0	10	5

(Continued)

[a]Multiple tests conducted during 1978-1979.

[b]Described in Part II: Methods and Materials.

[c]+++++ = amount of feeding on the control; ++++ = heavy feeding but less than control; ++ = 1/4-1/3 of feeding on control, plant not heavily damaged; + = nibbling - minor feeding, plant not damaged; 0 = no feeding or only an occasional feeding spot.

TABLE 1(Concluded)

PLANT FAMILY Plant Species	Common Name	Type of Tests	Relative Feeding Damage	Total No. of Beetles Tested	Total No. of Replications
PHYTOLACCACEAE					
Phytolacca americana L.	pokeweed	3	0	8	4
CARYOPHYLLACEAE					
Dianthus chinensis L.	China pink	1	0	8	4
CERATOPHYLLACEAE					
Ceratophyllum demersum L.	common coontail	1	0	12	3
POTAMOGETONACEAE					
Potamogeton illinoensis Morong	Illinois pondweed	1	+	30	5
P. nodosus Poir.	American pondweed	1	+	30	5
Potamogeton sp. (narrowleaved)	pondweed	1	0	12	3
CRUCIFERAE BRASSICACEAE					
Rorippa nasturtium-aquaticum (L.)	watercress	1	0	12	3

TABLE 2. LIST OF PLANTS ASSOCIATED WITH ACENTRIA NIVEA (OLIVIER) EITHER IN NATURAL HABITATS OR IN LABORATORY STUDIES. C = IMMATURES COLLECTED, R = IMMATURES REARED TO ADULTS, F = FED UPON IN LABORATORY

PLANT FAMILY Common Name	Scientific Name	Relationship	Record
CERATOPHYLLACEAE			
coontail	Ceratophyllum demersum L.	C	Berg (1942), Nigmann (1908), Personal*
		R	Treat (1955), Personal
		F	Batra (1977), Lekic & Mihajlovic (1970)
ELATINACEAE			
waterwort	Elatine americana (Pursh.)Arn.	F	Treat (1955)
HALORAGACEAE			
northern watermilfoil	Myriophyllum exalbescens Fernald	C	Batra (1977), Personal
		R	Batra (1977)
Eurasian watermilfoil	M. spicatum L.	C, F	Lekic & Mihajlovic (1970)
		R	Batra (1977), Personal
parrotfeather	M. aquaticum (Vell.)Verdc.	F	Personal
mermaidweed	Proserpinaca palustris L.	F	Personal
mermaidweed	P. pectinata L.	F	Personal
HYDROCHARITACEAE			
Brazilian elodea	Egeria densa Planchon	F	Personal
waterweed	Elodea canadensis Michx.	C	Berg (1942), Batra(pers.comm.)
		R	Berg (1942)
hydrilla	Hydrilla verticillata (L.fil)Royle	R	Batra (1977), Personal
NAJADACEAE			
southern naiad	Najas guadalupensis (Spreng.)Magnus	F	Personal
POTAMOGETONACEAE			
curly leaf pondweed	Potamogeton crispus L.	C	Ritsema (1878)
Illinois pondweed	P. illinoensis Morong	R	Personal
sago pondweed	P. pectinatus L.	C	Nigmann (1908)
pondweed	P. lucens L.	C	Gaevskaya (1969)
pondweed	P. gramineus L. (as P. heterophyllus Schreb.)	C	Nigmann (1903)
pondweed	P. perfoliatus L.	C	Berg (1942), Nigmann (1908)
pondweed	Potamogeton sp.	C	Berg (1942)
eelgrass	Zostera sp.	C	Nigmann (1908)
TRAPACEAE			
water chestnut	Trapa natans L.	C	Nigmann (1903)
ZANNICHELLIACEAE			
horned pondweed	Zannichellia sp.	C	Nigmann (1908)

* "Personal" denotes data from feeding tests in this study.

APPENDIX A: A LIST OF LOCALITIES WHERE LITODACTYLUS LEUCOGASTER HAS BEEN COLLECTED IN THE UNITED STATES AND CANADA. THE LOCALITIES WERE OBTAINED FROM A SURVEY OF MAJOR MUSEUMS

Locality	Date	No. of Specimens
CANADA		
Alberta		
Cypress Hills	5/25/1927	4
	6/25/1927	1
	6/27/1927	8
Cypress Hills, Provincial Park	6/17/1962	1
Cypress Hills, 5 miles* north of Provincial Park	6/17/1962	1
Edmonton	6/30/1915	1
Medicine Hat	July 1927	1
	6/18/1927	1
British Columbia		
Chaperon Lake	6/18/1932	1
Vernon	8/10/1929	1
Manitoba		
Le Pas	6/30/1917	1
Miami	8/5/1916	3
mile 214 H.B.R.	6/17/1917	1
	7/6/1917	1
St. Norbert	6/26/1907	?
Winnepeg	6/24/1911	1
	6/21/1917	?
Ontario		
George Lake, ca. 53°57', 114°06'	8/20/1973	1
Ottawa	?	?
Quebec		
Ile d'Anticosti, Port Menier	7/13/1973	1
Saskatchewan		
Sasu Regina, 1 mile east on Arcola St.	6/13/1969	2
UNITED STATES		
California		
San Francisco Co.	3/21/1915	1
San Mateo Co.	6/25/1966	1

(Continued)

* 1 mile = 1.609344 kilometres

APPENDIX A (Concluded)

Locality	Date	No. of Specimens
Georgia		
Decatur Co., 6.5 miles west of Bainbridge	5/24/1975	1
Johnson Co., 1 mile east of Wrightsville, Big Cedar Creek	9/18/1976	1
Illinois		
Lake Co., Fourth Lake	6/16/1892	1
Iowa		
Dickinson Co., Spirit Lake	June 1896	1
Hamilton Co., Jewell	Oct. 1936	1
Iowa Co.	6/28/1934	1
Story Co., Ames	7/14/1924	1
Minnesota		
Clearwater Co., 1 mile south of Lake Itasca	6/30/1964	1
Polk Co.	9/29/1959	1
Montana	?	1
Nebraska		
Thomas Co., Halsey	6/6/1967	1
North Dakota		
Billings Co., Kadramas Dam	?	1
Lake Teewaukon	7/15/1964	1
McHenry Co.	5/24/1963	1
Slope Co., Bowman	6/7/1965	1
Wells Co., James River	6/8/1964	8
	9/7/1964	3
Wisconsin		
Cedar Lake	7/14/1903	1
Dane Co.	5/11/1971	1
Winnebago Co., Oshkosh	?	1
Wyoming		
Carbon Co., N. Platte River, 7 miles east of Sinclair, 6700'	7/3/1964	1
Uinta Co., Evanston	7/3/1964	1

APPENDIX B: NORTH AMERICAN COLLECTION LOCALITIES OF ACENTRIA NIVEA. LOCALITIES WERE OBTAINED FROM THE LITERATURE AND PERSONAL COMMUNICATIONS

Locality	Date	No. of Adults Collected
CANADA		
Ontario		
Caledonia	7/16/1948	1
Hamilton, Dundas Marsh	6/6-8/15/1947	203
South Cayuga (Lake Erie)	7/12/1950	275
Quebec		
Lac 'a la Tortue	July 1947	1
Montreal	8/2/1927	1
	8/1/1944	1
	8/3/1944	1
White Lake	? 1977	?
St. Anne de Bellevue	July 1947	1
UNITED STATED		
Massachusetts		
Barnstable County, Barnstable	6/25/1949	1
	7/12/1950	2
	7/13/1950	1
	8/15/1954	1
Berkshire County, Tyringham	8/25/1953	100+
	9/1/1953	100+
	6/15/1954	22
	7/20-28/1954	83
	8/18-25/1954	171
Dukes County, Martha's Vineyard	8/3/1949	2
Plymouth County, East Wareham	7/24/1961	1
New York		
Oswego County, Minetto	6/22/1938	1
St. Lawrence County, Massena	8/5/1975	numerous
	9/17/1976	numerous
	6/19/1978	numerous
Wisconsin		
Dane County, Middleton	? 1963	?
Door County, Bailey's Harbor	8/5/1966	1

APPENDIX C: COMPARISON OF TWO METHODS OF REARING *LITODACTYLUS LEUCOGASTER* IN JARS

1. Two methods of rearing L. leucogaster in 0.94-1 glass jars were compared in order to determine if one was more productive. There were two sets of five jars with each set containing 3 females in three jars and 4 females in two jars. The females were left in the jars. In one set of five jars, flower spikes were added when necessary and the water was changed daily by pouring it through nylon organdy which covered the mouth of the jar. Fresh water was then added. Cocoons formed on the stems were allowed to remain submersed in the jar until the test was ended. In the second set of five jars, flower spikes were added at the same time as in the first set but the water was not exchanged. As the cocoons were formed, the stems were removed to jars containing wet paper toweling but little water. Thus the pupae developed in air instead of water. When the first new adults appeared, the test was discontinued and the living pupae were counted. The jars in which the cocoons were submersed produced $\bar{x} = 5.0 \pm 2.2$ (r = 2-8) living pupae, the jars with pupae in air produced 15.0 ± 3.4 (r = 10-19) living pupae. Many pupae died in the jar with the submersed stems. Although the removal of the cocoons increased the handling time and disturbed the larvae, it was necessary because the stems did not provide air to the cocoon. Even the number of pupae produced in the set in which the cocoons were removed was low compared to results obtained during earlier rearings when 40-60+ adults were obtained in some jars. The low number in this test may have been due to the quality of the flower spikes since the test was conducted at the end of the flowering period in November.

APPENDIX D: COMPETITION OF *L. LEUCOGASTER* AND *P. VESTITUS*

1. A series of tests was conducted in the large greenhouse cage to determine the result of competition between *L. leucogaster* and *P. vestitus*. Adults of each species were placed simultaneously and also sequentially into plexiglas cylinders containing milfoil flower spikes. Treatment 1 received two *L. leucogaster* females for one day, and then they were replaced by two *P. vestitus* for the second day. In Treatment 2 the order was reversed. In Treatment 3, two females of each species (4 insects) were placed together and not removed. Treatment 4 contained one female of each species (2 insects) which were not removed. Treatment 5 contained two *L. leucogaster* females for the duration and Treatment 6 contained two *P. vestitus* females for the duration. There were three replications of each treatment. The cages were checked after 20-22 days.

	No. *P. vestitus* cocoons	No. *L. leucogaster* cocoons
Treatment 1	3.3 ± 3.1 (r = 0-6)	1.7 ± 1.5 (r = 0-3)
Treatment 2	2.7 ± 0.6 (r = 2-3)	1.0 ± 1.0 (r = 0-2)
Treatment 3	1.0 ± 0.0 (r = 1)	5.3 ± 4.2 (r = 2-10)
Treatment 4	0	14.3 ± 7.2 (r = 6-19)
Treatment 5 (Control)	---	5.3 ± 2.9 (r = 2-7)
Treatment 6 (Control)	7.0 ± 5.2 (r = 1-10)	---

These results were too variable and the numbers too small, including the controls, to be meaningful but they do suggest that when *L. leucogaster* and *P. vestitus* are in constant competition for food that *L. leucogaster* possibly has an advantage. In nature, however, many factors would influence this competition. In these tests fungus on the flower spikes was probably responsible for the poor production of cocoons.

Figure 1. Eurasian watermilfoil infestation at Bagley Cove, Crystal River, Florida, during August 1979

Figure 2. Eurasian watermilfoil mat in flower at Bagley Cove, Crystal River, Florida, during August 1979

Figure 3. Flower spike of Eurasian watermilfoil

Figure 4. Cage in quarantine greenhouse used to rear Litodactylus leucogaster

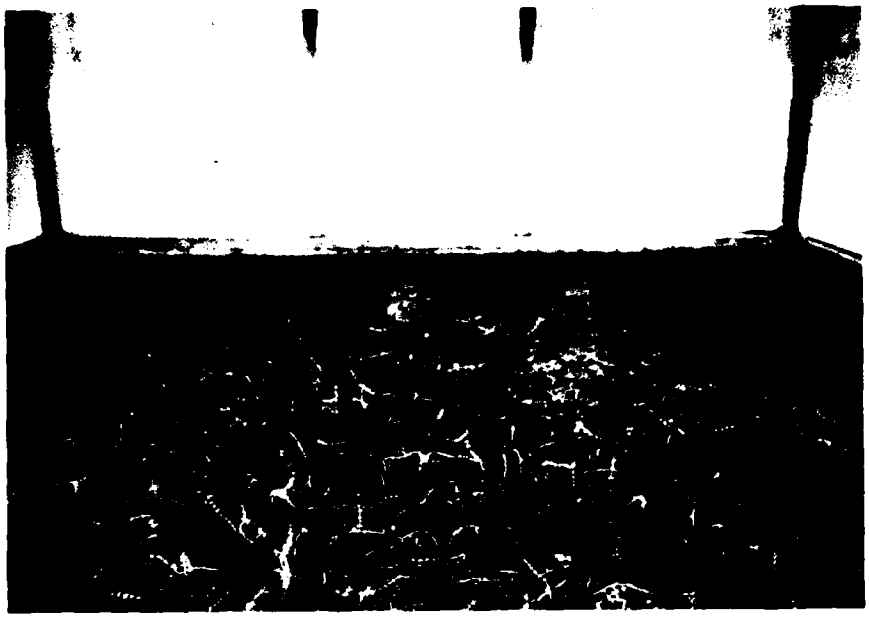

Figure 5. Eurasian watermilfoil inside greenhouse cage

Figure 6. Litodactylus leucogaster adult infected with the fungus Beauveria bassiana

Figure 7. Litodactylus leucogaster adult feeding on female flower of Eurasian watermilfoil

Figure 8. Eurasian watermilfoil flower spike damaged by feeding of Litodactylus leucogaster adults

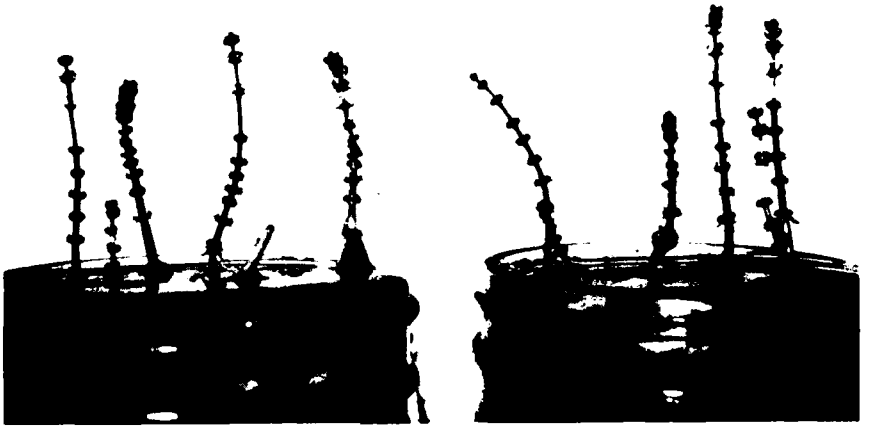

Figure 9. Eurasian watermilfoil flower spikes before exposure to Litodactylus leucogaster adults

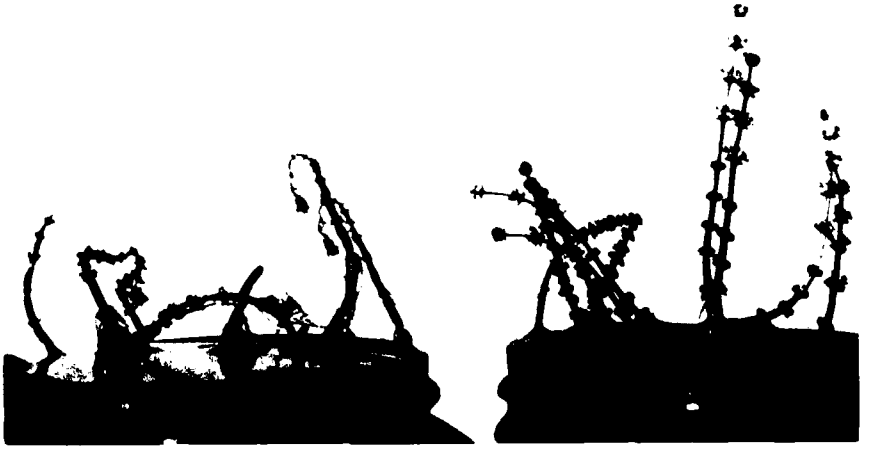

Figure 10. Eurasian watermilfoil flower spikes. Jar on left was exposed to ten adults for five days; jar on right was not exposed

Figure 11. Egg of Litodactylus leucogaster in ovary of Eurasian watermilfoil flower

Figure 12. First instar larva of Litodactylus leucogaster feeding on Eurasian watermilfoil flower

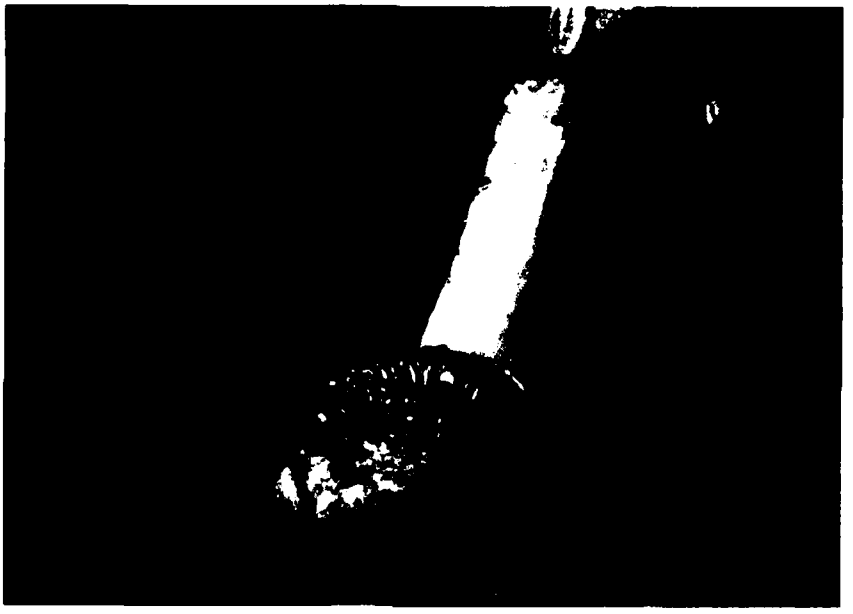

Figure 13. Mature larva of Litodactylus leucogaster feeding on Eurasian watermilfoil flower

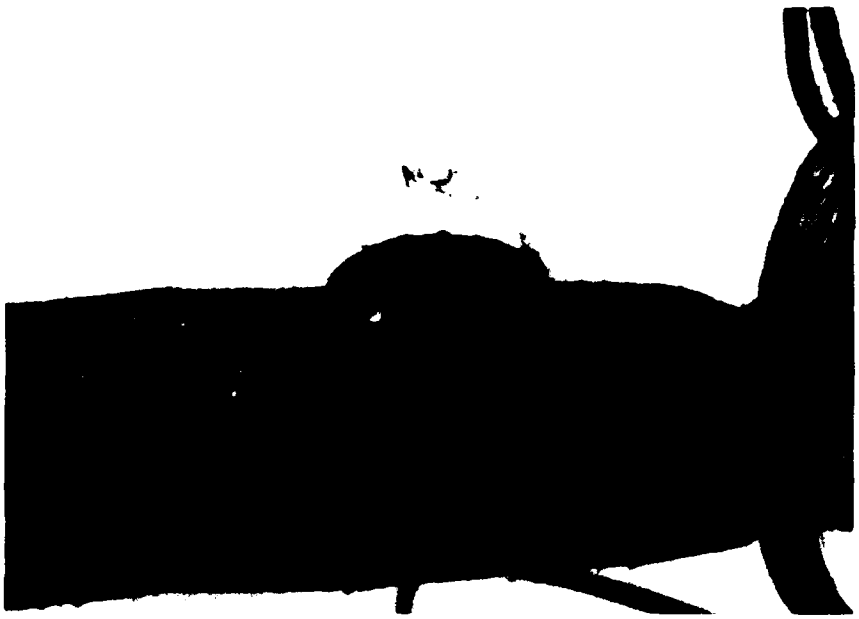

Figure 14. Cocoon of Litodactylus leucogaster in stem of Eurasian watermilfoil

Figure 15. Pupa of Litodactylus leucogaster in stem of Eurasian watermilfoil

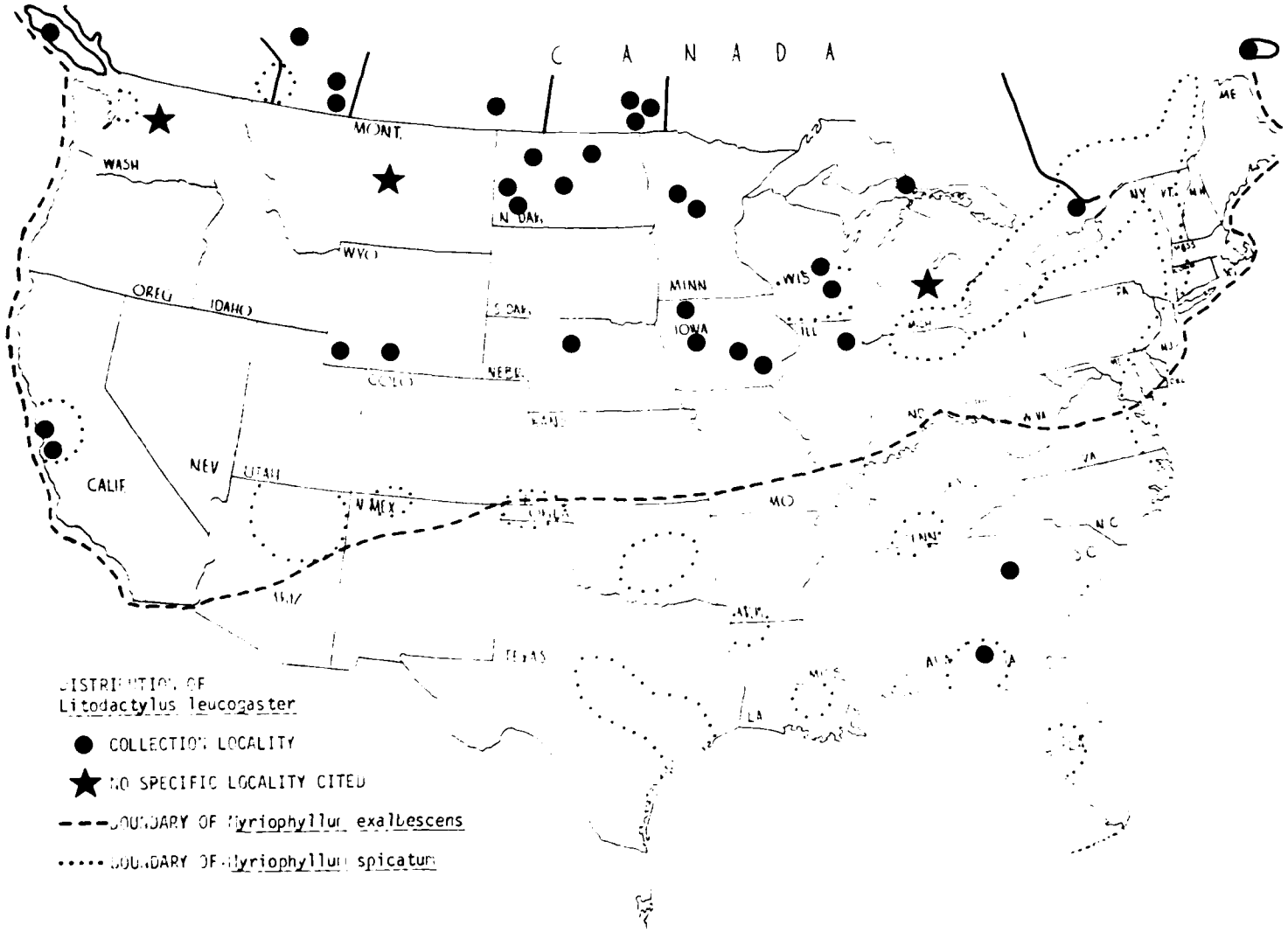

Figure 16. Distribution of Litodactylus leucogaster in North America

Figure 17. Release of Litodactylus leucogaster adults at Bagley Cove, Crystal River, Florida

Figure 18. Adults of the weevil species which attack Eurasian watermilfoil in the United States. L-R, Perenthis vestitus, Litodactylus leucogaster, Eubrichiopsis velatus

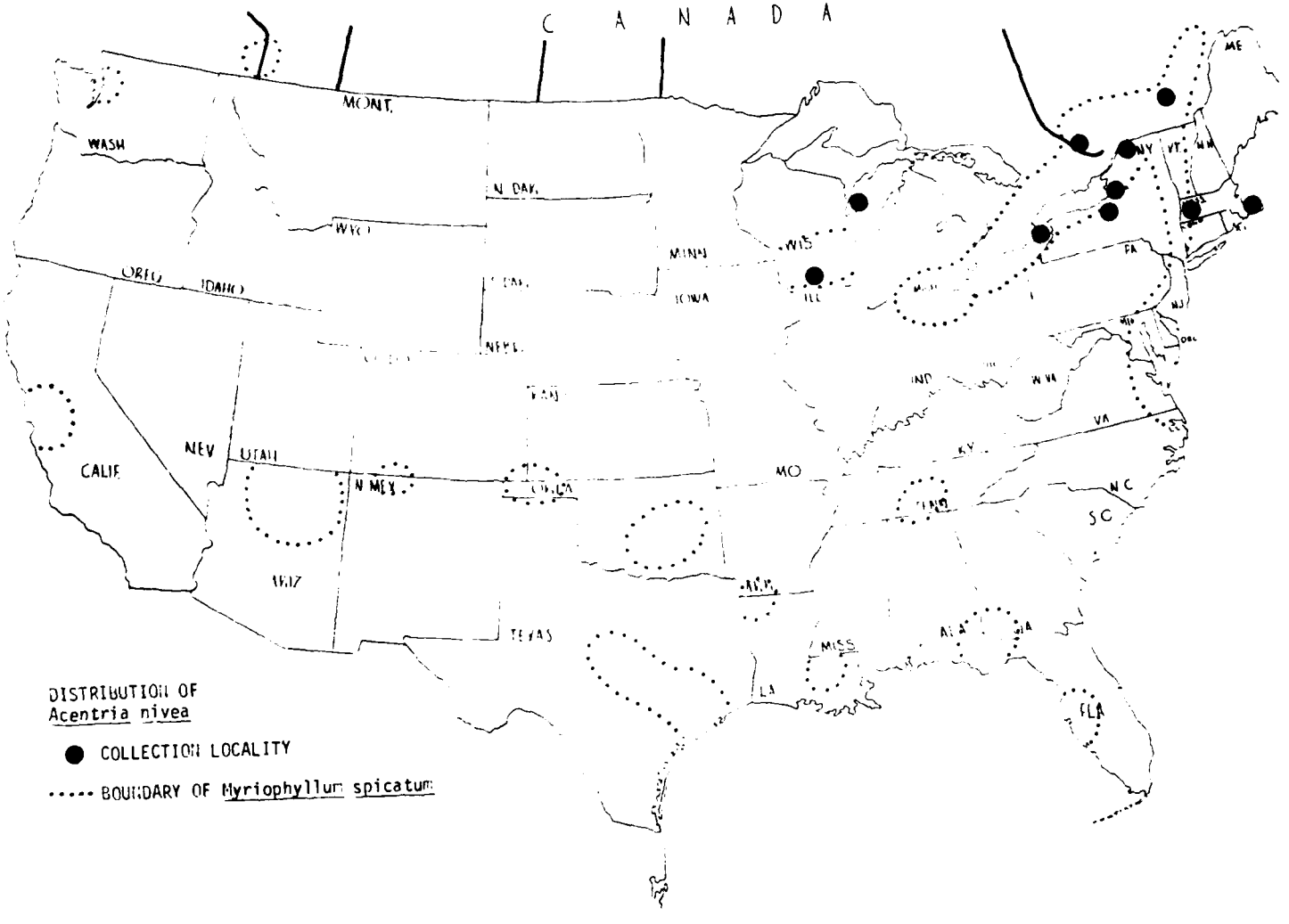

Figure 19. Distribution of Acentria nivea in North America

Figure 20. Acentria nivea male

Figure 21. Flightless Acentria nivea female

Figure 22. Young larva of Acentria nivea on Eurasian watermilfoil leaf

Figure 23. Mature larva of Acentria nivea on common coontail stem

Figure 24. Larva of Parapoynx sp.

Figure 25. Larval shelter of Acentria nivea on Eurasian watermilfoil

Figure 26. Mature larva of Synclita obliteralis on watervelvet, Azolla caroliniana.

Figure 27. Cocoon of Acentria nivea on Eurasian watermilfoil stem

Figure 28. Flightless Acentria nivea female ovipositing on submersed leaf of Eurasian watermilfoil (note single egg on leaflet between front legs)

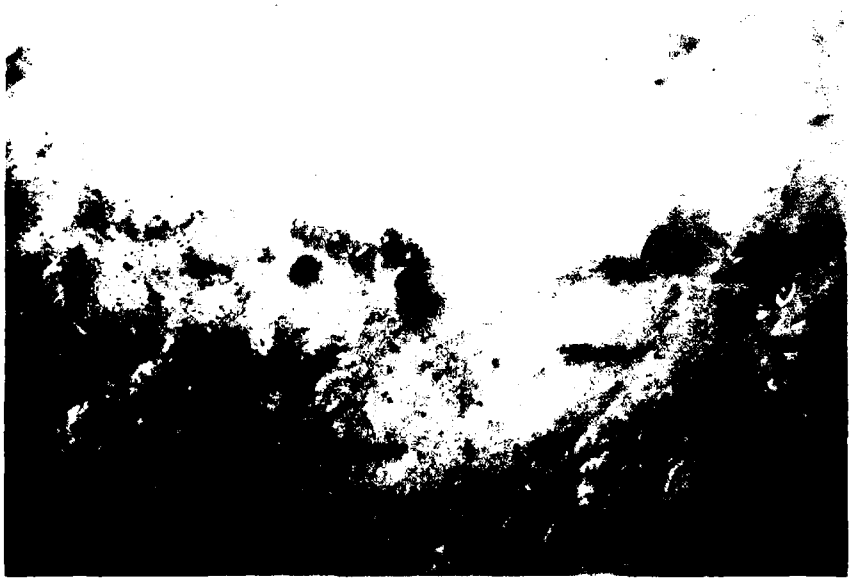

Figure 29. Eggs of Acentria nivea on a styrofoam float

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Buckingham, Gary R
Investigation of two insect species for control of Eurasian watermilfoil / by Gary R. Buckingham, Chris A. Bennett, Bonnie M. Ross, U.S. Department of Agriculture, Science and Education Administration, Gainesville, Fla. (Environmental Laboratory. U.S. Army Engineer Waterways Experiment Station) ; prepared for Office, Chief of Engineers, U.S. Army, Washington, D.C. -- Vicksburg, Miss. : U.S. Army Engineer Waterways Experiment Station ; Springfield, Va. : available from NTIS, 1981.
53, [24] p. : ill. ; 27 cm. -- (Technical report / U.S. Army Engineer Waterways Experiment Station ; A-81-4)
Cover title.
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References: p. 52-53.

1. Aquatic plant control. 2. Biological control. 3. Insects. I. Bennett, Chris A., joint author. II. Ross, Bonnie M., joint author. III. United States. Army Engineer Waterways Experiment Station. Environmental Laboratory. IV. United States. Department of Agriculture. Science and

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Education Administration, Gainesville, Fla. V. United States. Army. Corps of Engineers. Office of the Chief of Engineers. IV. Title: V. Series: Technical report (United States. Army Engineer Waterways Experiment Station) ; A-81-4.
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